Medical Library APR 7 1945

JOURNAL OF ANATOMY

ORIGINALLY THE JOURNAL OF ANATOMY AND PHYSIOLOGY

PAGE
137
145
157
177
180
187

CONDUCTED ON BEHALF OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND BY

A. B. APPLETON
J. D. BOYD
A. J. E. CAVE
W. J. HAMILTON
H. A. HARRIS
J. KIRK (TREASURER)
C. M. WEST
W. E. LE GROS CLARK (ACTING EDITOR)

VOLUME 79
PART 1
JANUARY 1945

Entered at New York Post Office as Second Class Matter
(All rights reserved)

CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE

CHICAGO: The University of Chicago Press
(Agents for the United States)
BOMBAY, CALCUTTA, MADRAS: Macmillan

All rights reserved

Price Twelve Shillings and Sixpence net

PRINTED IN GREAT BRITAIN

BIOCHEMISTRY AND MORPHOGENESIS

By JOSEPH NEEDHAM

324 illustrations. 52s. 6d. net

This book, though not a continuation of Dr Needham's earlier work, *Chemical Embryology*, is still concerned with the great problem of the relation between morphology and chemistry. Of possible approaches to this problem greatest attention is here given to the most recent line of research, the biochemical investigation of the morphogenetic hormones. The study of these chemical substances, of fundamental importance for all animal and plant development, including its hereditary aspect, occupies the greater part of the present book.

CAMBRIDGE UNIVERSITY PRESS

Large Department for
MEDICAL BOOKS

FOYLES
FOR BOOKS

New and Secondhand Books
on Every Subject
Nearly 3 Million Volumes
in Stock

BOOKS BOUGHT

119-125 CHARING CROSS
ROAD, LONDON, W.C.2

Tel. GERRARD 5660
(16 lines)

OPEN
9-6, Inc. Sats.

ORGANISERS & GENES

By C. H. WADDINGTON

2 plates. 14*s. net*

A new volume in the series of *Cambridge Biological Studies*

Development is a fundamental property of all animal structures and functions. The discovery of the organiser has in recent years opened the way to a much deeper causal analysis of development; and the science of genetics has shown that the factors which ultimately control developmental processes are the genes. This book attempts, firstly, to analyse the significance of the organiser phenomenon for the general theory of development, and secondly to exhibit the similarities between it and gene action. The first part of the book contains a survey of organisers in different groups, and a discussion of the concepts of evocation, individuation and competence, as three fundamental problems which are raised by the consideration of the organiser reaction. A discussion of the genetic data bearing on these problems leads to a consideration of the formation of animal shapes and patterns. The last chapter is devoted to an evaluation, in relation to the previous discussion, of some general biological concepts such as fields and organisation.

CAMBRIDGE UNIVERSITY PRESS

JOURNAL OF ANATOMY

THE REINNERVATION OF MUSCLE BY SENSORY NERVE FIBRES

By E. GUTMANN, *Department of Zoology and Comparative Anatomy, Oxford*

Denervation of a muscle is followed by its failure to contract and by the onset of atrophic changes. It is generally assumed that both contraction and the normal trophic state of a muscle depend upon the motor nerve fibres (Tower, 1931, 1939). However, it has been suggested that the trophic influence may be a function of sensory or sympathetic nerve fibres (see Cobb, 1925). Experiments in which a purely sensory nerve is made to reinnervate a previously denervated muscle can settle this question, as well as others related to the different properties of motor and sensory nerve fibres.

Such experiments raise two main questions, viz.: (1) Can sensory nerve fibres effect connexions with motor end-organs so that contraction can be produced by impulses passing distally along such nerve fibres? (clearly, since such impulses do not normally pass during life they can have no functional significance); and (2) Can the sensory nerve fibres, in spite of the absence of impulses, arrest or reverse in some manner the atrophy of muscle fibres?

In previous experiments of this type the two nerves commonly used have been the hypoglossal and the lingual. Earlier claims of contraction of the tongue by stimulation of the lingual nerve (Bidder, 1842; Heidenhain, 1883) were first criticized by Vulpian (1873). The lingual nerve is associated with the chorda tympani for a certain distance, and unless the chorda tympani fibres had been eliminated they themselves might have produced the functional response after union of the lingual with the hypoglossal nerve. Moreover, the lingual nerve contains secretory fibres and the hypoglossal is not a purely motor nerve (Langworthy, 1924). It appears, therefore, that these two nerves are not suitable for experiments on hetero-reinnervation. Langley & Anderson (1904) used the saphenous nerve as the central sensory stump and found no contraction of the muscles on stimulation after suturing it to the sciatic nerve. They concluded that afferent fibres are incapable of uniting with efferent fibres in such a way as to give motor effects. No histological observations were made, but the problem was clearly stated—'the observations do not exclude the possibility that afferent fibres may grow down the course of the efferent fibres and form afferent endings which from character or position are incapable of transmitting nervous impulses to muscle cells'. In this connexion the experiments of Weiss (1935), undertaken to test his theory of modulation of neurons by the muscles, are of interest. The central ends of spinal dorsal roots were sutured into transplanted muscles, thus producing a 'mononeural connexion'. Following electrical stimulation, contraction of the transposed muscles occurred in five cases out of fifteen. The fact that a functional test (sensory excitation of the afferent neurons) did not produce a motor effect, although electrical stimulation was effective, was taken as a proof of his 'resonance theory'. In any case, the positive effect of electrical stimulation suggested that sensory fibres had made functional connexions with the muscle fibres.

Even if it is not possible to produce contraction of a muscle by stimulating sensory fibres which have reinnervated it, it remains possible that the fibres may control the trophic state of the muscle. Thus Mott & Sherrington (1895) reported moderate wasting of muscles 4 months after posterior root section. However, Tower (1931) found no atrophic changes whatsoever, either macroscopically or microscopically, after ablation of the posterior root ganglia.

The morphological aspect of the hetero-reinnervation of muscle by sensory fibres was considered by Boeke (1913, 1917). The lingual nerve was sutured into the hypoglossal, and histological observations were made on the reinnervated muscle. The endings found in the tongue after this operation were reported to be somewhat irregular, but in general similar to the regenerated endings after simple end-to-end suture of the hypoglossal. Boeke states, 'Die regenerierenden Fasern, einmal in eine bestimmte Bahn eingedrungen, sind gezwungen in ihr weiter zu wachsen bis an das Ende', and, 'Der Habitus der Endveraestelungen der regenerierenden Nervenfasern wird durch die Umgebung bestimmt, die Nervenfaser verhaelt sich dabei ziemlich passiv'.

Histological observations on hetero-reinnervated muscles were also made by Weiss (1935), who claimed that 'atypical endings' were formed in the muscle, though he did not analyse these in detail.

The problem of hetero-reinnervation, that is to say, the reinnervation of a muscle by sensory nerve fibres, thus resolves itself into the following questions, viz.: (1) Is the form of each nerve ending determined by the nerve cell and central fibre? or (2) Is the anatomy of nerve endings determined by the reinnervated tissue? Boeke's findings provide evidence for the second possibility.

METHODS

The experiments personally conducted comprised studies of (1) reinnervation after immediate nerve suture, (2) reinnervation after delayed nerve suture.

(1) *Immediate suture.* In fifteen rabbits the peroneal and sural nerves were cut and the central stump of the sural nerve, a purely cutaneous nerve containing no somatic motor nerve fibres, was joined by plasma (Young & Medawar, 1940) to the peripheral stump of the peroneal nerve. The central stump of the peroneal nerve was resected as far as possible to avoid reinnervation of the peroneal muscles by its fibres. Experiments under this heading comprised the following three groups:

(*a*) Of seven animals in which the cross-union was performed on both sides. In two of these animals the distal stump of the peroneal nerve was dissected into its individual bundles and the sural nerve united with the branch to the m. peroneus longus: it was thought that a greater density of innervation would be achieved by this method, but no great advantage was observed.

(*b*) Of five animals in which the cross-union was performed on one side, while the contralateral peroneal nerve was cut and a long stretch of it resected. These experiments served to determine the effect of sensory reinnervation on the atrophy of denervation: the animals were killed 90 days after operation and the muscles on both sides weighed.

(*c*) Of three animals in which the cross-union was performed on one side, while on the other side an end-to-end union of the peroneal nerve was effected. In this way it was possible to make a comparison between hetero-reinnervated muscles and those reinnervated by motor nerve fibres.

Biopsy was performed at various times (53, 60 and 180 days) after operation.

(2) *Delayed suture.* In two animals the tibial nerve was cut and a stretch of it removed bilaterally: 20 months later a second operation was performed, whereby the sural nerve of one side was cut and its central stump united to the peripheral stump of the tibial nerve, whilst contralaterally the peroneal nerve was united to the peripheral stump of the tibial nerve. This experiment served to compare the pattern and the effect of reinnervation by motor and sensory nerve fibres in muscles denervated for long periods.

At the end of all the experiments the animals were anaesthetized and the sural nerve was stimulated faradically with bipolar electrodes. The muscles were also examined for the occurrence of fibrillation, and were then removed into a Petri dish, covered with gauze soaked in Ringer solution, dried with blotting paper and weighed. They were fixed in 10% formalin, and frozen sections of them were stained with a Bielschowsky modification (Weddell & Glees, 1941) or van Gieson.

RESULTS

(1) *Experiments on immediate suture.* In all animals a satisfactory junction of the nerve stumps was found at biopsy. Stimulation of the sural nerve produced no contraction of the muscles at whatever time after union this was done. However, pinching the peroneal nerve in the shank produced a nociceptive reaction, showing that reinnervation of the peroneal nerve with sensory nerve fibres had taken place in all cases.

In the animals left for longer periods the reinnervation of the peroneal nerve was also shown by the recovery of sensibility on the dorsum of the foot, i.e. the autonomous zone of the peroneal nerve (Gutmann & Guttmann, 1942). In one case in which the cross-union was performed on one side, while the contralateral peroneal nerve was cut and resected, a large sore developed on the completely denervated side. On the other hand, no such sore developed in the case in which the cross-union had been performed. Reinnervation with the sural nerve fibres had thus avoided, or made reversible, progressive atrophic changes of the denervated skin.

Fibrillation of the muscles innervated by sensory nerve fibres was clear in all cases where the cross-union had been performed, and no differences in the degree of fibrillation between reinnervated and completely denervated muscles were found. In four animals, in which the peroneal nerve was cut and resected on the control side, biopsy was performed 90 days after operation. The differences in muscle weight (in g.) were as follows:

Denervated side	1·4	1·8	1·0	1·3
Hetero-reinnervated side	1·3	1·8	1·0	1·0

Histological study showed no difference in the amount of connective tissue or in muscle fibre size.

The pattern of reinnervation. After suture of a motor nerve, contact between nerve and muscle fibres may be formed in two ways: (1) by reinnervation of the old end-plates, and (2) by formation of new endings by 'escaped' fibres (i.e. motor fibres which have failed to reach their old end-plates and run for long distances within the muscle), the pathways to the old plates having been obstructed by collagenization of the Schwann tubes and connective tissue increase between the muscle fibres (Gutmann & Young, 1944). Which of these types of connexion is formed depends largely on the duration of the denervation. The unions of the sural nerve with the peroneal nerve were done in the middle of the leg, and from the data on the rate of regeneration (Gutmann, Guttmann, Medawar & Young, 1942) the first regenerating fibres can be expected in the m. peroneus longus about 25–30 days after operation. This relatively short time of denervation should allow reinnervation of a considerable part of the old end-plates. Sections from

the m. peroneus longus taken 30 days after union showed a few thin nerve fibres only in the larger nerve trunks. Some empty end-plates were found, but at this time there were no nerve fibres near the end-plates. In a muscle taken 45 days after union the number of nerve fibres had increased and some were found in the smaller nerve trunks. Compared with the type of endings seen after a similar period of reinnervation by motor nerve fibres the endings seen were atypical. The sensory nerve fibres in the muscle ran for long distances along or across the muscle fibres (Text-figs. 1, 2) or in the connective tissue, frequently branching dichotomously, the branches crossing and recrossing one another and giving the whole ending the appearance of a network (Pl. 1, figs. 3–5, and Text-fig. 1). Although empty end-plates were frequent, they were rarely entered by the fine fibres. Yet in many cases the fibre must have grown down the tube towards the plate. In rare cases a fine branch was to be seen actually entering the plate (Pl. 1, fig. 6), but apparently the stream of axoplasm passed on without forming any of those large terminal branches within the plate such as are produced by motor nerve fibres (Text-fig. 2). When a sensory fibre arrives at an end-plate, it may show a thickening (Pl. 1, fig. 7, and Text-fig. 3), and a fine branch may arise and run over the surface of the plate (Pl. 1, fig. 7).

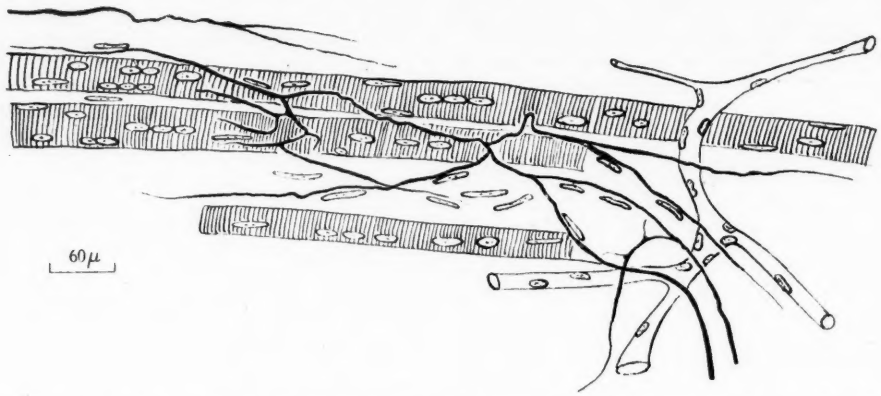
During their course the fibres tapered and thickened, apparently at points of obstruction, often branching here in several directions, in a fashion which may be termed 'stellate branching' (Pl. 2, fig. 8, and Text-fig. 4). The finest branches often ended in rings or bulbs, sometimes after crossing an end-plate, but these had no constant position (Pl. 2, fig. 9): they occurred at the muscle fibre, or near an empty end-plate, or within the connective tissue: it seems improbable, therefore, that such endings are important for the transmission of nervous impulses.

In muscle spindles the endings of the cutaneous nerve fibres on the intrafusal muscle fibres were similar to those formed on the extrafusal fibres. The nerve fibres entered the spindle and ran along the intrafusal muscle fibres forming a kind of network (Pl. 2, fig. 10). No annular endings, such as are found typically in the normal innervation pattern of proprioceptive fibres, were seen. The intrafusal fibres at this early stage were still intact but they clearly showed signs of denervation: this was specially indicated by the reaction of the nuclei which presented many nucleoli of various sizes (Pl. 2, fig. 11).

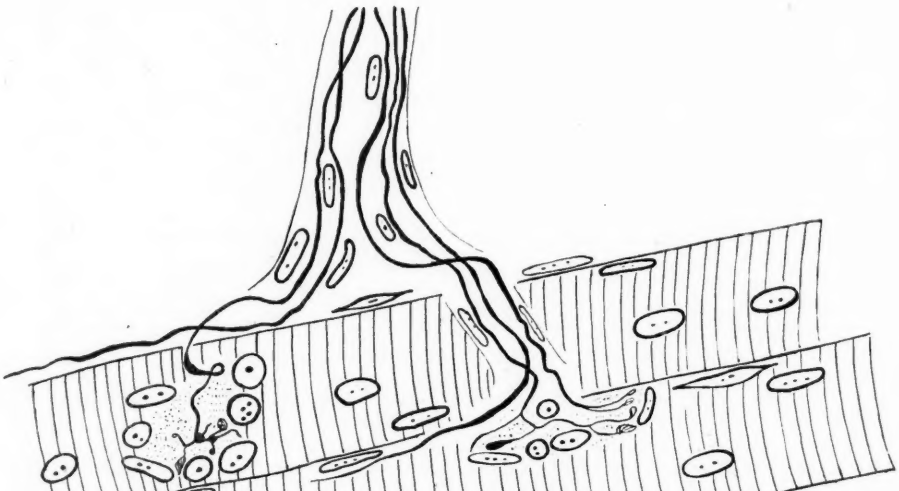
In preparations taken at later stages (77 days) after operation, similar pictures were found. Nerve fibres ran for long distances along the muscle fibres or within the connective tissue, which was increased (Pl. 2, fig. 12), and often stellate branching of the nerve fibres along the muscle fibres, as well as net-like endings, were observed (Text-figs. 1, 3 and 4). Neither innervated old end-plates nor newly formed end-plates were found. The picture of reinnervation at still later stages (180 days after operation) was on the whole similar to that found in earlier stages. Both net-like endings and stellate branching occurred: the former seemed to be of rarer occurrence than in the earlier stages, suggesting that some of the finest networks had been absorbed, but this cannot be stated with certainty on the basis of the material at present available. Most of the nerve fibres were still rather thin, but some had become thicker and even approached the diameter of motor nerve fibres. It is possible that these fibres were 'touch fibres', which have a larger diameter than 'pain fibres'.

The question arises to what extent are the endings found in the reinnervated muscles characteristic of sensory nerve fibres, and how abundantly can they be found after end-to-end suture also. In three animals an end-to-end suture of the peroneal nerve was made on one side and a cross-union with the sural nerve on the other. In animal 940 the muscles on the right side, where a simple suture had been made 53 days previously, showed a few net-like endings similar to those in the reinnervated muscles of the left side. Such findings are indeed to be expected after any nerve suture, on account of the growth of sensory fibres into motor pathways. The muscle reinnervated after direct suture also contained many innervated end-plates and 'escaped fibres'. These 'escaped fibres' can be distinguished from sensory fibres by their greater thickness and their direct course. They are absent from the hetero-reinnervated muscle. Animal 940 was examined 180 days after a similar bilateral operation. On the hetero-reinnervated side, atrophy had proceeded as in a denervated muscle. Some old end-plates were found, but no nerve fibres were seen running to them. Since sensory nerve fibres must at least sometimes have passed down the tubes leading to the plates, it must be presumed that any such had been removed later. On the control side, where a direct suture had been performed, occurred many innervated end-plates. There were no net-like endings resembling those found in early stages of hetero-innervation. 'Escaped fibres' were found, but they were thick and did not form net-like endings.

(2) *Delayed suture.* In the two animals in which delayed suture of the sural nerve into the tibial nerve on one side, and the peroneal nerve into the tibial nerve on the other side, was performed, the time of denervation was 20 months, the period of reinnervation, i.e. the time between arrival of fibres and biopsy, 6 and 8 months. Recovery of sensibility was extremely poor on both sides, responses to pin-prick being few and delayed, especially on the



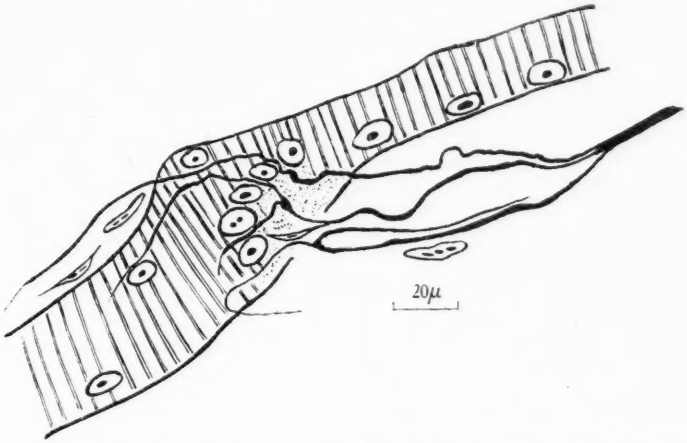
Text-fig. 1. Sensory nerve fibres forming networks along or between muscle fibres, some passing vessels and finally running within the connective tissue. No contact is made with the muscle fibres. 77 days after sural-peroneal nerve union (1003 A 1).



Text-fig. 2. Motor nerve fibres innervating two end-plates, one of the latter being shown in surface, the other in lateral view. The regenerating nerve fibres form terminal loops, thick end-bulbs, or tapering terminations between the end-plate nuclei. Two fibres escape and run on, one within the connective tissue, the other along the muscle fibre. 60 days after union of the cut peroneal nerve (940 A 1).

hetero-reinnervated side. The delayed responses may have been due to the thinner fibres in reinnervated nerve trunks which had been left denervated for a considerable time (see Sanders & Young, 1944). No recovery of motor function on either side was observed (as indicated by reflex spreading of first toe and elevation of the calcaneus). At biopsy stimulation of the sural nerve gave no

which consisted mostly of connective tissue and fat. Within the connective tissue lay the remains of the muscle fibres (Pl. 3, fig. 15)—sarcolemmal tubes filled with rows of nuclei, the latter mostly polygonal in shape with a foamy nucleoplasm. Small fragments of nucleoli were seen, but not the round and distinct nucleoli characteristic of normal muscle. The nerve fibres ran in long lines along the muscle



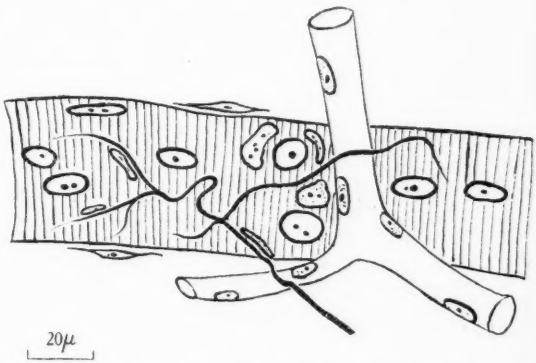
Text-fig. 3. Sensory nerve fibre apparently entering an end-plate. The nerve fibre shows no final branching at the end-plate and runs on along the muscle fibre towards the connective tissue. 90 days after sural-peroneal nerve union (784 C2).

contraction of the tibial muscles, whereas brisk contraction was observed on the control side where the peroneal nerve had been sutured into the tibial. Clear differences in the weight of the gastrocnemius and soleus muscles were found, as follows:

Animal no.	Peroneal into tibial, g.	Sural into tibial, g.
722	2·4	1·6
736	2·2	1·8

These differences indicate that the delayed reinnervation of the tibial muscles by peroneal nerve fibres had apparently stopped and reversed the atrophic changes to a certain extent, whereas no such change had followed the reinnervation with sensory fibres.

These indications were clearly supported by histological findings. The muscles of both sides displayed a considerable atrophy, portions of the muscle tissue being completely replaced by connective tissue. However, in those parts of the muscle in which recovery had taken place following reinnervation with motor nerve fibres, the muscle fibres were of large diameter and showed intact cross striation (Pl. 2, fig. 13). By contrast, the atrophy was much more conspicuous in the case of the hetero-reinnervated muscles (Pl. 2, fig. 14),



Text-fig. 4. Stellate branching of a sensory nerve fibre on a muscle fibre. 180 days after sural-peroneal nerve union (949 E 10).

fibres or across them (Pl. 3, figs. 16–18): most were rather thin (axon diameter less than 2 μ), but some were thick (axon diameter about 5 μ), and a few were not much thinner than motor fibres (Pl. 3, fig. 15). These last fibres were probably 'touch fibres', and their thickness is an indication that maturation of nerve fibres is apparently not dependent on the reinnervation of an end-organ but will take place without such a connexion. Net-like

endings were occasionally formed by thin nerve fibres, but this was a rare finding, indicating that these endings are resorbed in the later stages (Pl. 3, fig. 19).

The capsules of the muscle spindles showed no shrinkage, their size being apparently unaltered. The maintenance of this capsule may have been the reason why so often no atrophy of 'muscle spindles' has been assumed (see Tower, 1939). As in the case of extrafusal muscle fibres, net-like endings of the nerve fibres were rarely found during these later stages of reinnervation. The cutaneous nerve fibres entered the muscle spindles and ran in long lines through the 'lymphatic space' along extremely thin bands of muscle fibres (Pl. 3, fig. 20). No doubt atrophy of the intrafusal fibres set in after denervation and the cutaneous nerve fibres were unable to arrest the atrophic changes.

A different picture was shown on the control side, where the peroneal nerve had reinnervated the tibial muscles. The effects of long denervation were marked, and in a few parts sarcolemmal tubes filled with rows of nuclei indicated that atrophy had continued in spite of reinnervation (see Gutmann & Young, 1944) (Pl. 4, figs. 21, 22). The rather thick nerve fibres sometimes ran across quite thick muscle fibres so different from the extremely thin muscle fibres on the hetero-reinnervated side (Pl. 4, fig. 23). The nuclei approached normal structure: they were oval or even round, and contained distinct nucleoli. The nerve fibres were thick and had formed new end-plates (see Gutmann & Young, 1944) (Pl. 4, figs. 24, 25). The end-plates mostly stained somewhat darker than the other parts of the muscle fibre, and the site of neuro-muscular contact was indicated by the arrangement of the nuclei around the tapering terminal branches of the nerve fibres. Long 'escaped fibres' could also be found within the connective tissue, but they were mostly thick and often established new contact with the muscle fibres. Muscle spindles showed nerve fibres entering and running along the intrafusal fibres which, though rather thin, were better developed than those on the hetero-reinnervated side (Pl. 4, fig. 26).

It may thus be concluded that motor nerve fibres are able, if they make contact with muscle fibres, to arrest and reverse atrophic changes, even after very prolonged periods of denervation when the muscle fibres consist of thin threads only, packed with nuclear rows. After the establishment of new contact with the muscle fibres, recovery is indicated by increase of the diameter of the muscle fibres, decrease of connective tissue, and the return of the nuclei to their original shape and position. No such rearrangement follows reinnervation with sensory nerve fibres; rather, atrophy proceeds, leading to further disappearance of muscle fibres and to increase of connective tissue.

DISCUSSION

Motor nerve fibres innervating a muscle both reinnervate the old end-plates and form new end-plates (Gutmann & Young, 1944). Neither the innervation of old, nor the formation of new, end-plates was found after reinnervation with sensory nerve fibres. It appears, therefore, that sensory nerve fibres are unable either to establish contact with the sarcoplasmatic membrane of the muscle fibres or to arrest or reverse atrophic changes in a denervated muscle. (Hence contraction cannot be elicited by stimulation of sensory nerve fibres innervating muscle fibres.)

These findings are in agreement with the experiment of Langley & Anderson (1904) who obtained no muscular contraction upon stimulating the sciatic nerve, which had been reinnervated from the saphenous nerve. They contradict, however, the histological findings of Boeke and the experimental conclusions of Weiss. In the present experiments sensory nerve fibres were found at end-plates in the early stages of reinnervation, but no terminal thick branches occurred among the end-plate nuclei, as is the case with motor nerve fibres. Rarely the nerve fibre gave off a thin twig touching the end-plate, but the general result was that the fibres, instead of making contact with the plate, escaped and branched near it, without making close contact with the sarcoplasmatic membrane. Furthermore, sensory nerve fibres appeared unable to establish fresh contact with denervated muscles at any other points, in contradistinction to the faculty possessed by motor nerve fibres.

Boeke believed that the type of ending is decided by the 'milieu', so that a sensory fibre, once entering a motor tube, runs to the end-plate and innervates it. According to him, therefore, a sensory fibre can become morphologically a motor nerve fibre. No evidence for this was found in the present study. Sensory nerve fibres seem to have no power of entering into relation with muscle fibres; they form net-like endings and stellate branchings with configurations resembling the normal plexiform networks of sensory fibres in the skin. From such findings it appears that the type of ending of a nerve fibre is determined by the type of cell from which it originates. This view is supported by a further point. Most of the fibres, being 'pain fibres', remain thin, and even 6 months after suture they are much thinner than the corresponding fibres on the control side where motor fibres have been allowed to enter the muscle.

It seems, therefore, that an essential difference must exist between motor and sensory nerve fibres, accounting for their different behaviour. Either of the following factors may be responsible: (1) size of nerve cells and nerve fibres, (2) qualitative differences related to the nerve cell or fibre as

a whole, or in part. These factors may now be considered.

(1) Motor nerve fibres and nerve cells are larger than those of sensory neurons. Even long after reinnervation, sensory nerve fibres still remain thinner than motor fibres, indicating that the central stump or cell determines to a great extent the degree of maturation achieved (Sanders & Young, 1944). However, 'touch fibres' in the sural nerve may have a considerable diameter, though even these make no contact with the muscle fibres.

(2) There is a possibility that motor and sensory nerve fibres and cells differ in their chemical composition and metabolism, and such differences may be responsible for the fact that no contact can be effected between sensory fibres and muscle fibres, and that therefore no transmission of the nervous impulse to the muscle is possible. For example, differences in the rate or quantity of production of acetylcholine might account for the inability of sensory fibres to make contact with muscle fibres. Indeed, Loewy & Hellauer (1938) have shown that acetylcholine occurs in high concentration in the ventral spinal roots, whilst the dorsal spinal roots contain little or none. Moreover, synthesis of acetylcholine has been found to occur in motor but not in sensory roots (Feldberg, 1943). Macintosh (1941) determined the distribution of acetylcholine in certain nerves and found that those consisting mainly of motor fibres contain acetylcholine throughout their course, whilst those composed mainly of sensory fibres contain practically none. Nachmansohn (1939), on the other hand, found that the concentration of cholinesterase is of the same order of magnitude in both dorsal and ventral roots. Lastly, a difference in composition is indicated by the observations of Kwiatkowski (1943), who found high values of histamine in distal parts of sensory nerves, but little or none in motor nerves.

At present we can only conclude that the pattern of innervation in an end-organ is determined by either the size or the composition of the nerve cells from which the nerve fibres originate, although some secondary modification of the pattern may take place, probably in adjustment to the atrophic changes in the muscle.

SUMMARY

1. Hetero-reinnervation of muscles with sensory nerve fibres has been achieved by uniting the sural nerve with the peripheral stump of the peroneal nerve in rabbits.

2. The effects of denervation are not arrested by reinnervation of a muscle by sensory nerve fibres. Atrophy proceeds in the same manner as in a denervated muscle, even after sensory nerve fibres have entered the muscle, and fibrillation of the muscle does not cease.

3. Stimulation of the sural nerve does not produce contraction of the muscle fibres at any time after suture.

4. Sensory nerve fibres, even if they reach motor end-plates, are unable to make terminal branches, like those made by motor fibres; neither do they form new end-plates.

5. The endings of sensory nerve fibres in a muscle resemble those of sensory fibres in the skin. In the first stages of innervation they form many net-like endings and branch in a stellate manner. Few of these endings persist in the later stages of reinnervation; instead, the fibres run straight for long distances between the muscle fibres.

6. The pattern of innervation is apparently primarily determined by the type of cell from which the fibres originate, but may be modified in later stages of innervation.

7. Only motor fibres are responsible for the maintenance of 'trophic' conditions and only they are able to arrest and reverse atrophic changes of the muscle fibres.

The author is much indebted to Mr J. Z. Young for help and advice throughout the whole course of this work and for its revision. He also wishes gratefully to acknowledge a grant by the Nuffield Committee for the Advancement of Medicine. The work was assisted by a grant from the Rockefeller Foundation to the Department of Zoology and Comparative Anatomy, Oxford.

REFERENCES

Bidder, W. (1842). *Arch. Anat. Physiol., Lpz.*, **107**; quoted by Boeke (1917).

Boeke, J. (1913). *Pflüg. Arch. ges. Physiol.* **151**.

Boeke, J. (1917). *Verh. Akad. Wet. Amst.* **19**, no. 5, 1.

Cobb, S. (1925). *Physiol. Rev.* **5**, 518.

Feldberg, W. (1943). *J. Physiol.* **101**, 432.

Gutmann, E., Guttmann, L., Medawar, P. B. & Young, J. Z. (1942). *J. Exp. Biol.* **19**, 14.

Gutmann, E. & Guttmann, L. (1942). *J. Neurol. Psychiat.* **5**, 117.

Gutmann, E. & Young, J. Z. (1944). *J. Anat., Lond.*, **78**, 15.

Heidenhain, R. (1883). *Arch. Anat. Physiol.*, Suppl. Bd., p. 133.

Kwiatkowski, H. (1943). *J. Physiol.* **102**, 32.

Langley, J. N. & Anderson, H. K. (1904). *J. Physiol.* **31**, 365.

Langworthy, O. R. (1924). *J. Comp. Neurol.* **36**, 273.

Loewy, O. & Hellauer, H. (1938). *Pflüg. Arch. ges. Physiol.* **240**, 769.

Macintosh, F. C. (1941). *J. Physiol.* **99**, 436.

Mott, F. W. & Sherrington, C. S. (1895). *Proc. Roy. Soc.* **57**, 481.

Nachmansohn, D. (1939). *Bull. Soc. Chim. biol., Paris*, **21**, 761.

SANDERS, F. K. & YOUNG, J. Z. (1944). *J. Physiol.* (in the Press).
TOWER, S. S. (1931). *Brain*, **54**, 99.
TOWER, S. S. (1939). *Physiol. Rev.* **19**, 1.
VULPIAN, A. (1873). *C.R. Acad. Sci., Paris*, **76**, quoted by Boeke (1917).
WEDDELL, G. & GLEES, P. (1941). *J. Anat., Lond.*, **76**, 65.
WEISS, P. (1935). *J. Comp. Neurol.* **61**, 135.
YOUNG, J. Z. & MEDAWAR, P. B. (1940). *Lancet*, 2, 126.

EXPLANATION OF PLATES

(Animal reference number is given for each figure)

PLATE 1

Figs. 1, 2. Sensory nerve fibres running along muscle fibres and within the connective tissue: 45 and 90 days after sural-peroneal nerve union (845 B 3 and 784 C 2).

Fig. 3. Net-like endings of sensory nerve fibres innervating the m. peroneus longus: 45 days after sural-peroneal nerve union (845 B 3).

Figs. 4, 5. Details from fig. 3. Net-like endings of sensory nerve fibres innervating the m. peroneus longus.

Fig. 6. Sensory nerve fibre running towards an old end-plate but leaving again to run on in the connective tissue: 45 days after sural-peroneal nerve union (845 B 2).

Fig. 7. Detail from fig. 6. Sensory nerve fibre showing thickening at the end-plate and sending off a thin twig running over the surface of the plate.

PLATE 2

Fig. 8. Stellate branching of a sensory nerve fibre: 45 days after sural-peroneal nerve union (845 B 3).

Fig. 9. Sensory nerve fibre crossing a muscle fibre and forming end-rings (845 B 1).

Fig. 10. Cutaneous nerve fibre running along intrafusal muscle fibre (845 B 2).

Fig. 11. Detail from fig. 10. Intrafusal muscle fibres showing nuclear changes due to denervation.

Fig. 12. Sensory nerve fibre running along and across atrophic muscle fibres: 77 days after sural-peroneal nerve union (1003 A 3).

Fig. 13. Muscle fibres which have recovered following re-innervation with motor nerve fibres, after 20 months of denervation and 6 months reinnervation following peroneal-tibial nerve union (736 K 1). Compare the diameters and cross-striation of the muscle fibres with those in fig. 14.

Fig. 14. Atrophic muscle fibres, after 20 months of denervation and 6 months of reinnervation following sural-tibial nerve union (736 L 1).

PLATE 3

Fig. 15. Sensory nerve fibres running along highly atrophic muscle fibres without making contact. Note large diameter of some of the fibres ('touch fibres'?): 20 months of denervation and 8 months of reinnervation after sural-tibial nerve union (722 G 6).

Fig. 16. Sensory nerve fibres running along highly atrophic muscle fibres and branching within the connective tissue. The muscle fibres consist of sarcolemmal tubes filled with rows of nuclei: 20 months of denervation and 8 months of reinnervation after sural-peroneal nerve union (722 G 6).

Fig. 17. Sensory nerve fibres running for long distances between and along muscle fibres: 20 months of denervation and 8 months of reinnervation after sural-peroneal nerve union (722 G 6).

Fig. 18. Sensory nerve fibres running along atrophic muscle fibres without making contact: 20 months of denervation and 8 months of reinnervation after sural-tibial nerve union (722 G 6).

Fig. 19. Sensory nerve fibres reinnervating an atrophic muscle, forming a network within the connective tissue: 20 months of denervation and 8 months of reinnervation after sural-tibial nerve union (722 G 9).

Fig. 20. Sensory nerve fibre entering a muscle spindle and running through the 'lymphatic space': the short bands of nuclei are apparently the remains of the atrophic intrafusal muscle fibres: 20 months of denervation and 8 months of reinnervation after sural-tibial nerve union (722 G 5).

PLATE 4

Fig. 21. Motor nerve fibres reinnervating an atrophic muscle, and running for long distances between highly atrophic muscle fibres or within the connective tissue which has replaced some of these: note thickness of 'escaped' motor nerve fibres: 20 months of denervation and 8 months of reinnervation after peroneal-tibial nerve union (722 M 1).

Fig. 22. Motor nerve fibres reinnervating an atrophic muscle and running within the connective tissue and along remains of muscle fibres without making contact therewith: 20 months of denervation and 8 months of reinnervation after peroneal-tibial nerve union (722 M 1).

Fig. 23. Motor nerve fibres running across muscle fibres whose diameter is much thicker than in a muscle reinnervated by sensory nerve fibres only (compare figs. 15, 19): 20 months of denervation and 8 months of reinnervation after peroneal-tibial nerve union (722 M 4).

Fig. 24. Motor nerve fibres reinnervating an atrophic muscle and forming new end-plates. One branch escapes and runs on within the connective tissue: 20 months of denervation and 8 months of reinnervation after peroneal-tibial nerve union (722 M 1).

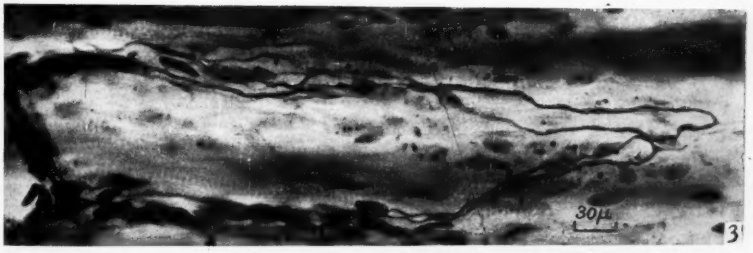
Fig. 25. Motor nerve fibres reinnervating atrophic muscle and forming new end-plates: 20 months of denervation and 8 months of reinnervation after peroneal-tibial nerve union (722 M 3).

Fig. 26. Motor nerve fibre entering a muscle spindle, running along and between the intrafusal muscle fibres which show a larger diameter than on the hetero-innervated side: 20 months of denervation and 8 months of reinnervation after peroneal-tibial nerve union (722 M 1).

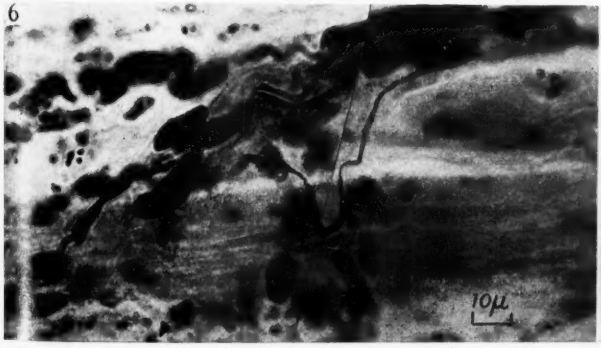
10μ
1

10μ
2

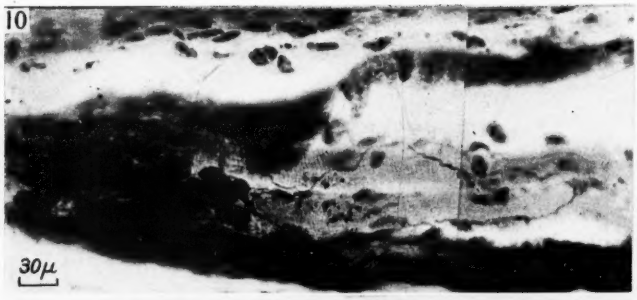
10μ
4

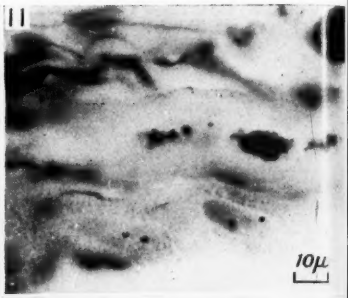

30μ
3

10μ
5


6
10μ

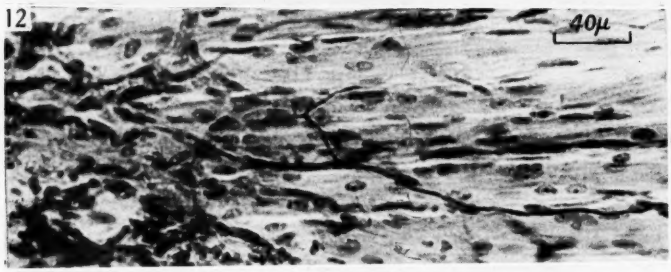
7

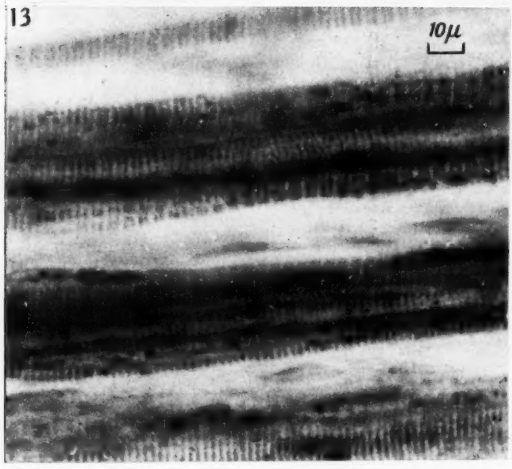

10
30μ

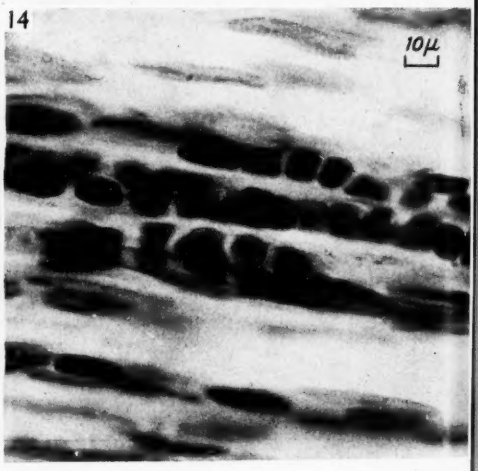

11
10μ

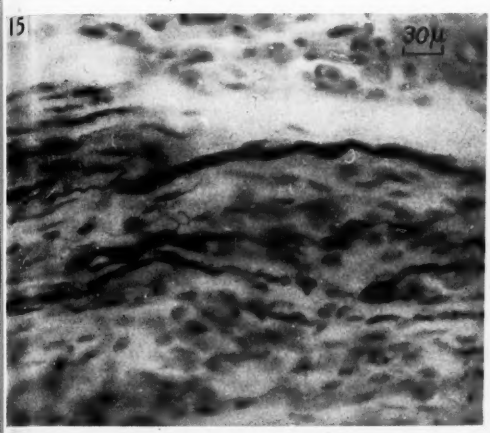
9
10μ

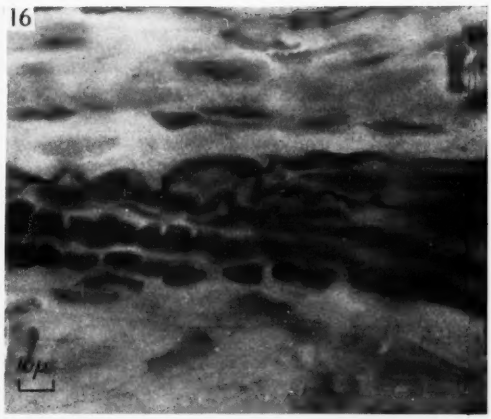
8
10μ

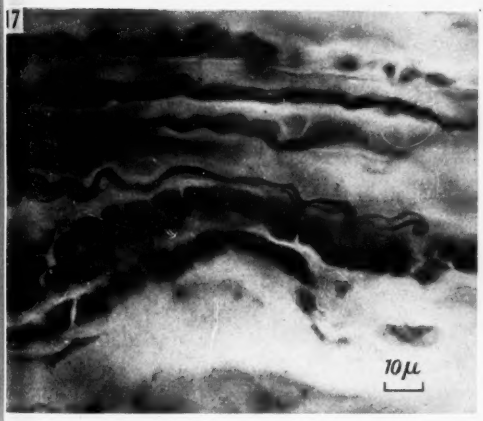

12
40μ

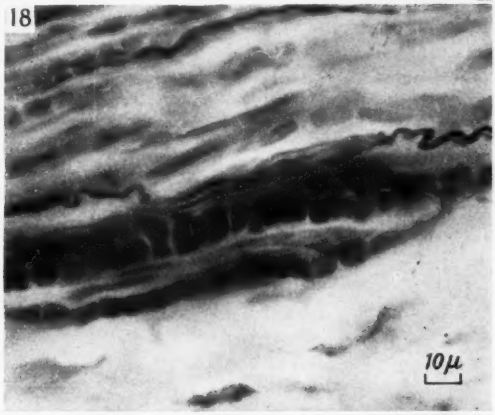

13
10μ

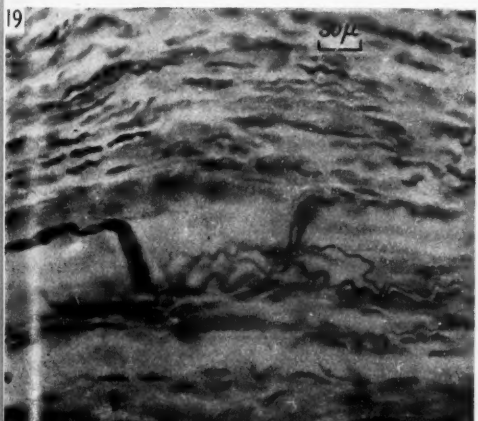

14
10μ


15
30μ

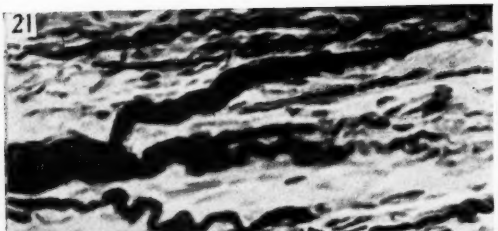

16
10μ

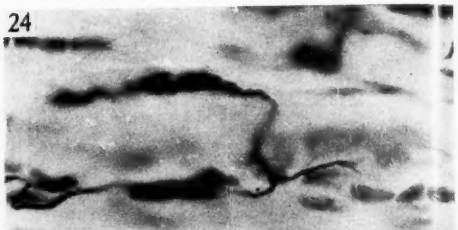

17
10μ

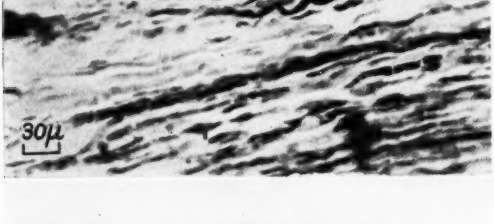

18
10μ


19
30μ

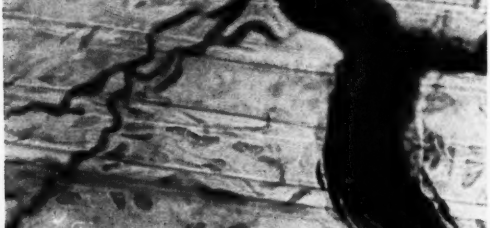
20
40μ


21
30μ


24
10μ


23
30μ

25
10μ


22
10μ

26
30μ

THE VASCULAR PATTERN OF PERIPHERAL NERVE DURING REPAIR AFTER EXPERIMENTAL CRUSH INJURY*

By P. BACSICH and G. M. WYBURN, *Department of Anatomy, University of Glasgow*

INTRODUCTION

'In end to end suture the surgeon must mobilize the nerve before performing any kind of resection. The central and peripheral stumps must be freed as far as is anatomically possible; most of the collateral nutrient vessels have to be divided but the longitudinal supply is so good that this is rarely harmful' (Seddon, 1943). Clinical evidence abounds to show that extensive mobilization of an injured nerve is not incompatible with successful repair. Moreover, Adams (1943) has demonstrated that after ligature of regional nutrient arteries to the sciatic nerve in the rabbit there are no harmful effects on structure or function. Either the peripheral nerves possess an alternative vascular supply capable of fully compensating for the loss of the regional arteries, or functional and structural integrity of the nerve is in large measure independent of its blood supply. This latter alternative had some support in the past as a result of confused thinking anent the doctrine of the trophic unity of the neurone, but there is now ample and formidable evidence, experimental and clinical (fully reviewed by Adams, 1942), to refute this assumption and insist on the necessity of an adequate blood supply for the proper functioning of any nerve. Circumstantial evidence, therefore, would seem to indicate that with the destruction of the regional nutrient arteries other vascular pathways can take over and maintain a sufficient blood supply.

The purpose of this work is to provide more direct evidence for this postulate by a study and comparison of the vascular pattern at different stages during the repair of an injured nerve where (1) there is no interference with the regional nutrient arteries, (2) the regional nutrient arteries are cut during extensive mobilization, and (3) in addition to treatment as in (2) all the visible longitudinal vascular channels are destroyed.

MATERIAL AND METHODS

Rabbits were used for the experiments, and the sciatic nerve exposed under nembutal anaesthesia, reinforced with ether. Following the usual lateral incision an adequate exposure was obtained by separation of the flexors and extensors without reflecting the origins of the femoro-coccygeus and glutei muscles. Haemorrhage was thus reduced to the minimum.

Two animals, generally litter mates of the same sex and approximately equal weights, were operated on at the same session. In one rabbit the sciatic nerve was crushed with Spencer-Wells forceps about midway down the thigh. This could be done with little or no disturbance of the position of the nerve. In the second animal the nerve was crushed and, while still gripped with the forceps it was widely mobilized and the regional nutrient arteries cut. These, as described by Adams (1943), include a proximal group from the inferior gluteal artery, an intermediate set from the perforating arteries, and a variable number distally ascending from the small saphenous artery.

After intervals varying from 3 hr. to 3 weeks a second operation was performed and from each pair of animals a portion of nerve about 1 in. long (including the crushed area) was removed and in addition in several animals a portion of the peripheral stump of the crushed sciatic nerve was taken out. The latter portion was examined with Nicol prisms, or stained by a modification of the Marchi method. The total degeneration of the myelin sheaths confirmed the efficiency of the crushing.

To eliminate genotypical differences, in the first two or three rabbits the sciatic nerve of each hind limb was exposed at the first operation period; the one crushed and the other crushed and mobilized. In these animals, with paralysis of both limbs, healing was often somewhat retarded with a tendency to trophic ulceration in the heel region and secondary infection. This method, although perhaps affording a more satisfactory basis for comparison, was therefore discarded.

In another set of rabbits the sciatic nerve on one side was crushed and mobilized as described above, and in addition the surface of the nerve was scraped with a Graefe cataract knife and all the visible longitudinal vessels destroyed above and below the crushed area. Sometimes it was necessary to cut into the epineurium to ensure interruption of a somewhat more deeply situated longitudinal channel. The animals were left for periods varying from 4 to 14 days when a portion of the devascularized nerve was removed.

After removal, the crushed portions of the sciatic nerves were fixed and stained by a modification of Pickworth's benzidine method devised to prevent the fragmentation of the sections and to enable us where necessary to cut sections serially. With this modification the blood vessels stain the usual blue-black, the nerve tissue yellow, and

* This work was undertaken at the suggestion of the Peripheral Nerve Sub-Committee of the Medical Research Council.

the connective tissue a rust brown, without the use of any counterstain. All the sections were cut at 100 μ.

As we have previously stated (Bacsich & Wyburn, 1940), we consider that the benzidine method possesses certain advantages in the study of vascular architecture. Benzidine stains only the blood in the functionally open capillaries and thus gives a true picture of the actual physiological state of the circulation at the time. Injection methods, on the other hand, to be successful, apply considerable pressure, force open all potential vascular pathways, and present the vascular pattern at its maximum capacity, a condition seldom attained under normal circumstances. The benzidine staining is therefore peculiarly suited to the study of adaptive changes in vascular pattern under varying conditions, while injection methods should be reserved for purely anatomical investigations.

DESCRIPTION

A full description of the blood supply of the sciatic nerve in the rabbit is given by Adams (1943) and we, in large measure, agree with his findings. On the surface of the nerve, visible to the naked eye, are uninterrupted longitudinal vascular trunks situated for the most part in the surface layers of the epineurium (Pl. 1, fig. 1) and not interfascicular as stated by Adams (1943). Because of their superficial position they can be destroyed by light scraping of the surface of the nerve. Interfascicular branches are given off from the epineural arteries (fig. 1), and these form a secondary longitudinal system with numerous cross-branches on the perineural surface of the fasciculi. An occasional branch from an epineural artery may pierce a fasciculus (Pl. 1, fig. 2). From the perineural vessels branches penetrate into the nerve bundles (Pl. 1, fig. 3)—intrafascicular arteries—where they form a tertiary system of longitudinal channels (Pl. 1, fig. 4) visible microscopically as a network showing numerous anastomoses and dichotomous branching. It is very improbable that during normal functioning of the nerve this intraneural vascular plexus is opened up and completed to the extent suggested by the results of injection methods. Our fig. 4 resembles the fig. 3 of Adams (1943), whose description is based on studies following injection of Indian ink. But our fig. 4 from animal no. 4 (which was regarded as unsuitable for the study of changes in the vascular pattern) represents a section from the central end of an injured and extensively mobilized nerve where at the second operation there was evidence of inflammatory change with consequent dilatation of blood vessels. In the normal nerve, sections of the same thickness (100 μ) stained by the benzidine method, show only fragments of sparsely spaced capillaries (Pl. 1, fig. 5). We consider this figures the true functional condition of the intraneural vascular network. The capillary pattern is subject to constant change, and capillary continuity is interrupted by alternating opening and closing of segments. The open segments containing blood are the stained fragments, and these frequently show as a linear arrangement of evenly spaced black dots; each dot is a red blood corpuscle occupying the calibre of the capillary.

Comparison of the vascular pattern of the crushed nerves with that of the crushed and mobilized nerves

(1) *After* 24 *hr. Animals nos.* 5*b and* 8*b*

Naked eye appearance. There was no difference between the two nerves. In both cases there was a haemorrhagic plate occupying the width of the crushed area. Here, as in most of the other specimens, the central and peripheral stumps adjoining the crushed part have a characteristic rounded appearance.

Microscopic appearance. There is no significant difference between the two nerve sections. In both, the haemorrhagic plate is a prominent feature. There is an increased vascularity, better marked in the central end, and a dilatation of blood vessels in the areas adjacent to the crushed zone. Blood vessels bridge the crushed area and penetrate into the haemorrhagic plate (Pl. 1, figs. 6, 7).

(2) *After* 48 *hr. Animals nos.* 10 *and* 11.

Naked eye appearance. In both nerves the crushed area was occupied by a haemorrhagic plate and there was no detectable difference in the vascularity.

Microscopic appearance. The vascular picture in the two sections is markedly similar. There are some slight differences. In the unmobilized nerve the haemorrhagic plate is rather more extensive and there is a greater vascularity of the central stump as contrasted with a greater vascularity of the peripheral stump in the mobilized nerve. In both cases a number of blood vessels penetrate and traverse the haemorrhagic area (Pl. 1, figs. 8, 9).

(3) *After* 3 *days. Animals nos.* 12*b and* 13*b*

Naked eye appearance. The haemorrhagic plate was present in both nerves.

Microscopic appearance. The haemorrhagic plate is more prominent in the mobilized nerve (cf. 48 hr. specimen) while in the unmobilized nerve there are rather more dilated blood vessels. Otherwise there is no marked difference between the two sections (Pl. 1, figs. 10, 11).

(4) *After* 4 *days. Animals nos.* 10*b and* 7*b*

Naked eye appearance. No difference. Haemorrhagic plate present in both.

Microscopic appearance. There is a larger haemorrhagic plate in the unmobilized nerve. The vascularity is approximately the same in the two nerves (Pl. 1, figs. 12, 13).

(5) *After* 7 *days. Animals nos.* 6*b and* 9*b*

Naked eye appearance. The haemorrhagic plate was not conspicuous in the unmobilized nerve.

Microscopic appearance. The haemorrhagic plate, especially in the unmobilized nerve, is incomplete and gives the impression that already some absorption has taken place. In the mobilized nerve numerous large vessels can be seen crossing the haemorrhagic plate. In both nerves there is greater vascularity of the central stump. There is no significant difference in the vascular picture of the two nerves (Pl. 2, figs. 14, 15).

(6) *After* 14 *days. Animals nos.* 12 *and* 13.

Naked eye appearance. There was no haemorrhagic plate in either the mobilized or unmobilized nerve, but in both there was a relatively greater vascularity of the crushed area.

Microscopic appearance. The two sections are strikingly similar. There is complete absence of haemorrhagic plates but in tangentially cut sections of both nerves there can be observed an epineural vascular mantle composed of a close network of blood vessels (Pl. 2, fig. 18). There is considerable vascularity, better marked in the central stump where in addition to the normal longitudinal channels there are a number of obliquely running branches—possibly ingrowths from the periphery (Pl. 2, figs. 16, 17).

(7) *After* 3 *weeks. Animals nos.* 18 *and* 18*b*

Naked eye appearance. No haemorrhagic plates, but there is an increased vascularity of crushed area.

Microscopic appearance. It is difficult to distinguish between the sections of the two nerves. The crushed areas are not, as in the earlier specimens, a prominent feature of the sections, but have become more a part of the general picture, while the adjoining ends have lost the characteristic 'neuroma' appearance (Pl. 2, figs. 19, 20). In tangentially cut sections the epineural vascular mantle is already considerably reduced both in size and density.

Nerve 3 *hr. after crushing* (*unmobilized*). *Animal* 3*b*

Naked eye appearance. Typical appearance of central and peripheral stumps already present. The crushed area shows a marked constriction. There is no haemorrhagic plate.

Microscopic appearance. The crushed area, clearly seen, is crossed by a few slightly dilated vessels. There is a relative hyperaemia of the 'ends' adjacent to the crushed area where a number of the vessels end abruptly, and in the central stump there are a few small haemorrhages (Pl. 2, fig. 21).

Nerve 3 *months after crushing* (*unmobilized*). *Animal no.* 6

Naked eye appearance. The nerve as a whole was somewhat thinner and the crushed area occupied by a fusiform swelling, rather reminiscent of the 'callus' of a healing fracture (cf. earlier specimens).

Microscopic appearance. The fusiform swelling includes a number of tortuous blood vessels. In the central and peripheral stumps the vascular pattern is approximating to the normal (Pl. 2, fig. 22).

Nerves which have been crushed, mobilized, and the surface scraped to destroy the epineural longitudinal channels

After 4 *days. Animal no.* 15*b*

Naked eye appearance. Well-marked constricted crushed area. There was no haemorrhagic plate.

Microscopic appearance. There is a number of dilated blood vessels adjacent to the crushed part, particularly noticeable in the central stump. At the periphery vessels bridge the crushed area (Pl. 2, fig. 23).

After 7 *days. Animal no.* 11*b*

Naked eye appearance. The crushed area was not so prominent as in animal no. 15*b*.

Microscopic appearance. There is a small haemorrhage in the centre of the crushed part but no haemorrhagic plate. There are considerably fewer blood vessels (Pl. 2, fig. 24) than in animal no. 15*b*.

After 10 *days. Animal no.* 17*b*

The appearances are very similar to those described in animal no. 11*b*. There are a number of blood vessels and a small haemorrhage in the crushed area. Otherwise there is a still further decreased vascularity (Pl. 2, fig. 25).

After 14 *days. Animal no.* 16*b*

There are relatively few vessels in the crushed area itself, but the general picture as compared to animals nos. 11*b* and 17*b* is one of greatly increased vascular activity (Pl. 2, fig. 26) and resembles very closely figs. 16, 17, 19 and 20 on Pl. 2. There are a few obliquely running blood vessels indicating vascular invasion from the surrounding tissues.

DISCUSSION

A comparison of the vascular pattern of the regenerating sciatic nerve at intervals up to 3 weeks after (*a*) a simple crush injury, and (*b*) where the crushing is combined with extensive mobilization and cutting of the nutrient arteries in the thigh region, makes it quite clear that it would not be possible to distinguish between the mobilized and unmobilized nerves on the basis of any consistent difference in the vascularity. Differences, of course, exist and could be anticipated as individual characteristics. For example, the haemorrhagic plate is more extensive in the unmobilized nerve after 48 hr., while after 3 and 4 days the haemorrhagic plate is more prominent in the mobilized nerves. Of more interest than the variations in the extent of the haemorrhagic plate is the fact that in both sets of experiments it does not appear until some time between 3 and 24 hr. and has disappeared at 14 days.

The significant fact is not these fluctuating differences but rather the essential similarity of the vascular picture in both the mobilized and unmobilized nerves.

If, as our experiments would seem to indicate, cutting the regional nutrient arteries causes little upset or alteration in the vascularity of the nerve, it can only be inferred that the longitudinal channels, now the main source of blood supply, are equal to the task of maintaining the normal blood flow.

In considering the question of the importance of the longitudinal channels as an effective collateral supply Adams (1942) states: 'In spite, however, of the importance which has always been attached to the longitudinal anastomosis in this respect its significance appears to have been considerably overestimated for it must be emphasized that anatomically the blood supply of nerves is fundamentally regional and thus it is very probable that each nutrient artery dominates the blood supply of its own particular territory of nerve.' Later in the same work (p. 335) Adams again stresses that the nutrient arteries cannot be regarded as mere reinforcing vessels for the longitudinal channel and finds the *coup de grâce* of any such view in its implication 'that devascularisation of a nerve along its length by removing the regional source of supply would in no way impair its nutrition'. This implication receives considerable justification from his later experiments, when, following ligature of the nutrient vessels, no histological changes were detected in nine out of twelve nerves. Our results would certainly lead us to suggest that the longitudinal vascular pathway can at least claim the status of equal partnership with the nutrient arteries and the apparent facility with which it can cope with the extensive deprivation of the regional source of supply would seem to deprecate any undue emphasis on the territorial dominance of the nutrient collaterals. A similar view is expressed by Young (1942): 'Many blood vessels enter the trunks along their length but the supply extends for great distances so that removal of all vessels entering over a considerable stretch of nerve may not seriously affect its function.'

The experiments of Gutmann (1942), which showed that ligature of the popliteal artery and vein did not delay recovery of the crushed peroneal nerve in the rabbit, may indicate the importance of the longitudinal channels in the maintenance of an adequate blood supply to the nerve.

When, in addition to extensive mobilization, the visible longitudinal vessels are cut there is no haemorrhagic plate, and in the early stages (animals nos. 15*b*, 11 and 17*b*) the nerve sections are anaemic. After an interval of 14 days (animal no. 16*b*) the circulation is largely restored, and it is presumed that this has been accomplished by collateral pathways provided mainly by the secondary and tertiary longitudinal system. Thus as a result of the destruction of the longitudinal blood vessels there is some delay in the establishment of a normal circulation, and this early period of ischaemia may act as a delaying factor in the subsequent regeneration, although Toracca (1920), after mobilizing and stripping the epineural sheath of the sciatic nerve of the dog (which would destroy the longitudinal vessels), reports that the only functional disturbance was a transitory paralysis. This may well have been due to trauma or possibly occurred during the short period of ischaemia before the blood supply was restored to normal. Toracca concluded that the intrinsic blood supply of a nerve could fulfil its functional requirements.

Haemorrhagic plate

The occurrence of haemorrhage into the injured part of the nerve some time between 3 and 24 hr. is probably due to the recovery and dilatation of blood vessels around this area. During the first 14 days the blood clot is absorbed. The course of events is the same in the mobilized and unmobilized nerves, and it is a remarkable testimony to the adequacy of the longitudinal channels that the onset and extent of the haemorrhage is not affected by destruction of the regional nutrient arteries.

Some degree of adhesion to surrounding structures, usually the muscles, was present at the second operation at the later stages, particularly in mobilized nerve (animals nos. 13 and 18*b*), and it is therefore not impossible that there is some vascularization of the nerve from extraneural blood vessels. We consider that the obliquely running blood vessels which are a conspicuous feature in the nerve sections of animals nos. 12, 13 and 16*b* (14 days after crushing) and 18 and 18*b* (3 weeks after crushing) are ingrowths from vessels of the neighbouring tissues.

The fact that extensive mobilization of a nerve does not seem to delay clinical recovery or interfere with the physiology of the uninjured nerve was perhaps responsible for a tendency to belittle the importance of the blood supply to peripheral nerves and to create an impression that axons, as long as they maintained their continuity with the parent cell, were not influenced by diminished blood supply. This apparent indifference of the nerve, however, is easily understood when it is appreciated that even with widespread loss of regional nutrient arteries the longitudinal channel can furnish a normal blood supply. There is much evidence to indicate that nerves subjected to procedures which produce an ischaemia, not necessarily complete, exhibit serious functional disturbance. Bentley & Schlapp (1943) tested the conductivity of the sciatic nerve in the cat after interference with the blood supply. They found (*a*) after cutting the regional nutrient vessels

that 'the longitudinal vessel sufficed to maintain at a high level the excitability of the nerve', and (*b*) after cutting the regional nutrient vessels and the longitudinal vascular channel that conduction was lost in the nerve in the upper third of the thigh. The normal state of the nerve distally is due, they say, to the blood supply ascending from the leg along the longitudinal vessel. Bentley & Schapp have shown that the effects of ischaemia are primarily due to the fall in the supply of oxygen below the level necessary for nerve conduction.

Our results, however, do not encourage us to agree with the further conclusion of Bentley & Schlapp that the circulatory requirement of a nerve is small. In the mobilized nerve we could detect no change in the vascular picture which would suggest diminution in the amount of blood circulating through the nerve. This implies that the vascular disposition is such that a normal blood flow can be sustained in face of the loss of much of the usual source of supply—a somewhat superfluous precaution if the interpretations of Bentley & Schlapp are correct. On the other hand, where there is evidence of decreased circulation, as in the first 2 weeks after destruction of the longitudinal vessels, Bentley & Schlapp record a loss of conduction.

The ischaemic paralysis and anaesthesia produced by the experiments of Lewis, Pickering & Rothschild (1931) afford a convincing demonstration of the effects of interference with the blood supply of the peripheral nerves. Lewis *et al.* describe a centripetal anaesthesia which can be produced by the use of a special clamp exerting pressure on a stretch of nerve while permitting blood circulation distally and a delayed but uniform and simultaneous onset of anaesthesia where there is more direct pressure of a short stretch of nerve such as the ulnar at the elbow or peroneal at the neck of the fibula. The authors suggest that the centripetal type of anaesthesia occurs when the ischaemia is complete, while if the ischaemia is incomplete the onset of anaesthesia is simultaneous throughout the areas distal to the pressure zone. In our opinion a more probable explanation is that the pressure on the ulnar and peroneal nerves directly against bone is sufficient for complete local ischaemia and consequent nerve block with a uniform onset of anaesthesia. With the use of the pneumatic cuff and the special clamp the ischaemia is incomplete—there is no nerve block but conduction fails first distally as a result of the more rapid development of anoxia in the leg.

Other work on the functional effect of loss of blood supply is reviewed by Adams (1942).

Perhaps the tardy recognition of the indispensable role of the vasa nervorum might in part be ascribed to the greater allure of the so-called trophic influence of the nerve cell on all its appendages and the compelling interest of the degenerative changes which follow interruption of the continuity of axon and cell body. It is perhaps unfortunate that the term trophic is so generally applied to the power of the cell body over its processes. It is difficult to conceive that the nerve cells of the spinal cord, for example, could be directly concerned in the conveying of nourishment to the distal parts of the axons of the long nerves of the limbs. The axons are nourished, as is recognized by most neurologists, through the neurilemma cells which, like the cells of the nucleated capsules in the posterior root ganglia and the neuroglia of the central nervous system, act as nurse cells and feed the axons with nourishment obtained from the blood vessels. Some such function is implied by Cajal (1928) when he states in connexion with nerve degeneration 'it looks as though the destruction of the conducting filament were a signal for the emancipation of the cells of Schwann from their nutritive function'. The question is also discussed by Bielschowsky (1927): 'Die modernen Untersuchungsmethoden haben zwar zu dem Ergebnis gefuhrt, dass den Gliazellen und den ihnen aquivalenten Schwannschen Zellen ein Betrachtlicher Einfluss auf die Ernahrung der Ganglienzellfortsatze eingeraumt werden muss'; and regarding the influence of the nerve cell body on its processes: 'Da diese Eigenschaft der Ganglienzelle im wesentlichen dynamischer Natur ist und von den ortlich wirkenden Faktoren der gliogenen Elemente zum mindesten begrifflich getrennt werden kann, ist es ganz zweckmassig, an Stelle der trophischen Einheit nach dem Vorgange Heidenhains (1911) die histodynamische zu setzen.' 'Histodynamic' seems to us a happier description of the force which maintains the integrity of the axon and creates the functional bond between it and the nerve cell—a force which is probably of the same nature as the dominance of the nucleus over its protoplasmic territory. Young (1944) advances the view that the influence of the nucleus and cell body is the maintenance of a previously arranged orientation of protein particles in the axon. This it does by the transmission along the axon from the cell of a 'turgor pressure'. Isolation of the nerve produces disorientation as shown by the contracted coils and spirals of the isolated axons.

The conclusions which emerge from the observations recorded here are: (1) that the vascular arrangement of a peripheral nerve is such that its blood supply is not adversely affected by extensive mobilization, and (2) after destroying not only the regional nutrient arteries but also the longitudinal anastomoses the circulation of the nerve appears to be restored to normal within a maximum period of 2 weeks. These can only attain their full significance when correlated with a study of growth rate of regeneration. With this object in view further experimental work is at present in progress,

and if it can be shown, as legitimate inference from the preliminary results would lead us to expect, that, even fairly extensive interference with visible vasa nervorum does not seriously affect the rate of regeneration, there will be an anatomical basis for the clinical anticipation of success following surgical procedures involving gross disturbances in position and normal relations of injured peripheral nerves.

SUMMARY

1. The vascular pattern of a nerve injured by crushing is compared with that of a nerve which has been crushed and extensively mobilized with destruction of the regional nutrient vessels.

2. The comparative study of the vascular pattern is made at intervals from 24 hr. to 3 weeks after injury.

3. There is no significant or constant difference between the vascular patterns of the mobilized and unmobilized nerves nor any evidence of quantitative diminution of circulation after cutting the regional nutrient arteries.

4. The longitudinal vascular channels are able to maintain an adequate and apparently normal circulation.

5. A study was made of the vascular pattern of nerves which had been crushed, mobilized with destruction of regional nutrient arteries, and the longitudinal channels destroyed. In the early stages there is definite evidence of ischaemia, followed by a return to the 'normal pattern' 14 days after the initial injury.

6. The results furnish corroborative evidence and support the claim that gross disturbance of the position of a peripheral nerve involving considerable interference with its blood supply is not necessarily detrimental to successful regeneration.

The expenses of this research were met by a grant from the Rankin Medical Research Fund. We wish to thank Prof. D. M. Blair for reading the manuscript and making many helpful suggestions.

REFERENCES

ADAMS, W. E. (1942). *J. Anat., Lond.*, **76**, 323.
ADAMS, W. E. (1943). *J. Anat., Lond.*, **77**, 243.
BACSICH, P. & WYBURN, G. M. (1940). *Trans. Roy. Soc. Edinb.* **79**, 60.
BENTLEY, F. H. & SCHLAPP, W. (1943). *J. Physiol.* **102**, 62.
BIELSCHOWSKY, M. (1927). *Handb. Mikr. Anat. Mens.* (Mollendorff), **4**. Berlin.
CAJAL, S. R. (1928). *Degeneration and Regeneration of the Nervous System.* London.
GUTMANN, E. (1942). *J. Neurol. Psych.* **5**, 81.
LEWIS, T., PICKERING, G. W. & ROTHSCHILD, P. (1931). *Heart*, **16**, 1.
SEDDON, H. J. (1943). *Lancet*, **2**, 671.
TORACCA, L. (1920). *Chir. Organi Mov.* **4**, 279.
YOUNG, J. Z. (1942). *Physiol. Rev.* **22**, 318.
YOUNG, J. Z. (1944). *Nature, Lond.*, **153**, 333.

DESCRIPTION OF PLATES

All the photomicrographs are sections of sciatic nerve of the rabbit cut at 100 μ and stained by the benzidine method. *C.* = crushed; *C.M.* = crushed and mobilized; *C.M.S.* = crushed, mobilized and stripped. Unless stated otherwise magnification is ×18.

PLATE 1

Fig. 1. Normal nerve. Epineural vessel on surface of sciatic nerve—note trichotomous branching and cross-connexions with smaller longitudinal perineural vessel. ×10.
Fig. 2. Normal nerve. Perforating branch of epineural vessel. ×10.
Fig. 3. Normal nerve. Intrafascicular vessel. ×40.
Fig. 4. Intrafascicular longitudinal network from animal no. 4. *C.M.* ×40.
Fig. 5. Normal nerve. Intrafascicular longitudinal network. ×40.
Fig. 6. Animal no 5*b*. *C.* 24 hr.
Fig. 7. Animal no. 8*b*. *C.M.* 24 hr.
Fig. 8. Animal no. 10. *C.* 48 hr.
Fig. 9. Animal no. 11. *C.M.* 48 hr.
Fig. 10. Animal no. 12*b*. *C.* 3 days.
Fig. 11. Animal no. 13*b*. *C.M.* 3 days.
Fig. 12. Animal no. 10*b*. *C.* 4 days.
Fig. 13. Animal no. 7*b*. *C.M.* 4 days.

PLATE 2

Fig. 14. Animal no. 6*b*. *C.* 7 days.
Fig. 15. Animal no. 9*b*. *C.M.* 7 days.
Fig. 16. Animal no. 12. *C.* 14 days.
Fig. 17. Animal no. 13. *C.M.* 14 days.
Fig. 18. Animal no. 12. *C.* Epineural vascular mantle around crushed area. ×15.
Fig. 19. Animal no. 18. *C.* 3 weeks.
Fig. 20. Animal no. 18*b*. *C.M.* 3 weeks.
Fig. 21. Animal no. 3*b*. *C.* 3 hours.
Fig. 22. Animal no. 6. *C.* 3 months.
Fig. 23. Animal no. 15*b*. *C.M.S.* 4 days.
Fig. 24. Animal no. 11*b*. *C.M.S.* 7 days.
Fig. 25. Animal no. 17*b*. *C.M.S.* 10 days.
Fig. 26. Animal no. 16*b*. *C.M.S.* 14 days.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10.

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Fig. 16

Fig. 17

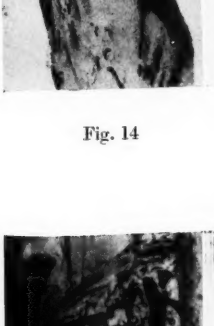


Fig. 18

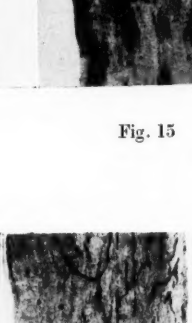


Fig. 19

Fig. 20

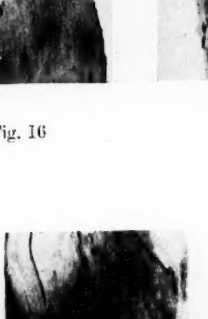


Fig. 21

Fig. 22

Fig. 23

Fig. 24

Fig. 25

Fig. 26

BACSICH AND WYBURN—VASCULAR PATTERN OF NERVES

THE EFFICIENCY OF INTRAMUSCULAR ANASTOMOSES, WITH OBSERVATIONS ON THE REGENERATION OF DEVASCULARIZED MUSCLE

By W. E. LE GROS CLARK and L. B. BLOMFIELD, *Department of Anatomy, University of Oxford*

A considerable amount of work has been done on the effect on muscles of the occlusion of the main limb arteries and veins (e.g. Brooks, 1922, 1924; Brooks & Martin, 1923; Jepson, 1926; Holman & Edwards 1927; Wilson, 1932; Brooks, Johnson & Kirtley, 1934; and Griffiths, 1940). These experimental observations have shown that in the absence of a collateral circulation such a procedure leads to necrosis of the muscular tissue, sometimes in the form of scattered patches and sometimes producing a massive sequestrum. Moreover, the effect is evidently produced quite rapidly. An early observation by Heidelberg (1878) showed that the application of a tourniquet to the hind-limb of a rabbit leads to muscle necrosis if it is left on for 24 hr. Garré (1906) produced necrosis after only 1 hr. of devascularization, and Griffiths (1940) has concluded, on the basis of clinical and experimental studies, that the whole damage to a muscle following upon devascularization is produced within a few hours, or even minutes. As regards the effects of devascularization of individual muscles by interfering locally with their blood supply, there is little information in the literature. We may refer to the work of Brooks (1922) who isolated the rectus femoris in dogs, though his results hardly accord with those of other observers, and are very difficult to evaluate. Thus he states that in two cases in which the muscle was completely freed from all vascular connexions (including those which reach it through its attachments) it showed no pathological change at the end of periods of 40 and 110 days. In another similar experiment, the muscle is actually stated to have completely disappeared in 21 days, apparently without leaving any trace. Middleton (1930) reported experiments on the sartorius muscle in dogs, in which he interrupted the venous drainage while leaving the arterial supply intact. This procedure led, within 8 weeks, to a generalized interfibrillar fibrosis and contracture of the whole muscle.

No direct studies appear to have been made on the effects of partial devascularization of muscles and the efficiency of intramuscular vascular anastomoses. The question is clearly of importance in relation to the muscular changes which result from injuries involving muscles and their vascular supply, not only from the point of view of functional recovery but also because of the complications which may ensue in wounds containing devascularized tissue. In war wounds, for example, injured muscles are particularly prone to infection with anaerobic organisms, and it seems probable that this is facilitated by a temporary impairment of blood supply resulting from interruption of vascular channels (see Campbell & Pennefather, 1919; Trueta, 1943). It is well known that individual muscles are commonly supplied by two or more main vessels, in addition to small subsidiary vessels which may reach them through their attachments, and it has been generally assumed that the anastomotic connexions within each muscle are sufficiently free to permit of adequate vascularization even if one or other of the channels of supply is interrupted. Spalteholz, who studied the vascular pattern in muscles in a classical monograph in 1888, expressed some doubts on this point, for, although he demonstrated abundant arterial anastomoses, he suggested that in many cases they were probably too fine to allow an adequate collateral circulation in the case of a sudden occlusion of one main vessel.

In a short paper, published at the end of the last war, Campbell & Pennefather (1919) concluded, from a radiographic study of human muscles of which the arteries had been injected with a light bismuth salt, that the muscles can be divided into three main groups: those with a blood supply derived from many different sources and in which potential anastomoses are quite numerous, those with a blood supply from only two or three different sources in which the anastomoses are relatively few, and those in which the blood supply is derived from only one source. In view of the fine calibre of the anastomoses, they even suggested that arteries in muscle are as much 'end-arteries' as those in the brain.

In view of the practical considerations which depend on the efficiency of intramuscular anastomoses, we have undertaken an experimental study of this problem by attempting to assess the degree to which vascularization of muscular tissue is affected by interruption of vessels of supply or intramuscular vessels, and by gun-shot wounds.

METHODS

In all experiments rabbits were used. The muscles selected for special investigation were tibialis anterior, semimembranosus and gracilis. In each case a preliminary study of the vascular supply was made by dissection, and of the intramuscular vascular pattern by preparing specimens injected with India ink and cleared in oil of wintergreen. As might be

expected, the vascular pattern varies to some extent in the same muscle in different animals, both in regard to the relative size of vessels of supply and the details of their branching within the muscle, and this serves to explain variations which have been found in the effects of interrupting certain vascular channels. The main features of the vascular pattern, however, appear to be fairly constant. Ligature of individual vessels and interruption of intramuscular channels were carried out under ether anaesthesia and with the usual aseptic precautions. The gunshot wounds were made with the assistance of our colleague, Dr Delisle Burns. In these experiments, rabbits under nembutal anaesthesia were shot through the adductor region of the thigh with a steel bearing $\frac{3}{32}$ in. in diameter, at a velocity varying from 250 to 1000 m. per sec.

In order to assess the effects of these procedures on the vascularity of muscles, we have made use of a highly diffusible dye, bromo-phenol blue, which was employed by Rous & Gilding (1929) as an indication of the ability of the circulation to serve the tissues under various conditions. It was subsequently used by Rous, Gilding & Smith (1930) in their studies of the gradient of vascular permeability, and by Gilding (1932) in his investigations on the course of vaso-constrictor nerves to the periphery.

Bromo-phenol blue is an innocuous dye which has the advantage of being without effect on the heart and blood vessels in the pure state, of reaching the tissues very rapidly, and of staining them so distinctively that even faint intensities are easily detected. Its diffusibility through water (as determined by Rous, Gilding & Smith) is approximately half that of dextrose but about twice that of dyes such as trypan blue and trypan red which are commonly used for vital staining. Following the technique of the investigators already quoted we have employed an isotonic 4% solution of bromophenol blue, to the amount of 3 c.c. per kg. weight. The dye was injected over a period of approximately 3 min. into the ear vein. At the end of a further 4 min. the animal was killed by decapitation to allow rapid exsanguination. In such experiments, the normal muscles of the rabbit are found to be stained a bright blue colour (except in the case of 'red' muscles such as the tenuissimus, where the colour is obscured by muscle pigment). The dye is apparently adsorbed by the myosin of the muscle fibres (and is not merely located in the tissue spaces of the endomysium), for it has proved resistant to ordinary extractives such as saline, acids, alkalis and alcohol when the tissue is thoroughly pulped. It can, however, be extracted with pyridin, and this method may perhaps be available for quantitative estimations. In teazed preparations of muscle fragments from animals injected with bromo-phenol blue, the colour is too faint to be detected under the microscope (even when compared with normal tissue with the use of comparison eyepieces and when higher concentrations of the dye have been injected). If, however, a teazed preparation of fresh muscle is treated on the slide with a drop of 4% of the dye for a few seconds and then washed thoroughly with saline for some time, the contractile substance of the muscle fibres is found to be well stained, while the nuclei, endomysium and connective tissue elements of the muscle appear quite unstained. The fact that the dye is taken up by the muscle fibres themselves makes it of particular value for evaluating the physiological efficiency of the circulation, since it provides an indication of the degree of interchange between the capillaries and muscular tissue even where a feeble circulation in the main blood vessels may in fact be present. Clearly it is this aspect of the vascularity of tissues which is primarily of practical importance in the study of the effects of interference with vessels of supply. Rous & Gilding have stated that, as far as the skin is concerned, the circulation had to all intents and purposes ceased to be effective in those areas which bromo-phenol blue had failed to penetrate in their experiments, and, as we shall see, the same applies to muscular tissue.

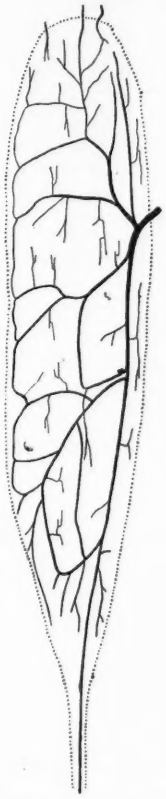
Many of the muscles used in our experiments were subsequently prepared for histological study by sectioning in paraffin and staining with haemotoxylin and eosin.

THE VASCULARIZATION OF THE TIBIALIS ANTERIOR MUSCLE

Vascular pattern. In the rabbit, the tibialis anterior receives two main vessels of supply from a common trunk which is derived from the anterior tibial artery. They enter the upper half of the muscle close together on its deep surface and near its medial border. The upper vessel is directed proximally and supplies approximately the upper third of the muscle, while the lower branch descends to vascularize the lower two-thirds. In addition, some very small vessels enter the muscle through its upper attachment to the tibia, and a fine vessel accompanies the tendon of insertion. The vascular pattern, as demonstrated by the injection of a muscle with India ink, is shown in Text-fig. 1. It will be noted that the main vessels extend longitudinally close to the medial border of the muscle, giving off a series of primary branches which are directed obliquely distally and laterally. These, again, give off secondary branches which, by anastomotic connexions, form a series of arcades along the lateral margin of the muscle.

Ligature of one main vessel of supply. In a series of rabbits, the lower artery of supply was ligatured (together with its accompanying vein), and the effects on the vascularity of the lower two-thirds of the muscle observed by the injection of bromophenol blue. If the animal is injected with the dye

immediately after the operation, the upper or proximal part of the muscle becomes stained a bright blue while the lower or distal part in most cases remains completely unstained, the line of demarcation between the two parts being commonly quite distinct (Text-fig. 2*a*). The precise position of the line of demarcation varies in different animals, presumably in relation to variations in the intramuscular vascular pattern. Two days after a similar operation the distal part of the muscle is usually still found to be unstained after injection of the dye (Text-fig. 2*b*), though in one case it assumed a very faint tinge of blue with scattered patches of uncoloured muscle tissue. In another experiment, the lower vessels were ligatured 4 days before injection, and in this case the blue staining indicated that most of the muscle had been revascularized from above. In the lower third of the anterior surface an oval area could be demarcated in which the staining was less intense than normal (Text-fig. 2*c*). Longitudinal section of the muscle showed macroscopically, however, that most of its substance in its distal half was well coloured, and in patches even more intensely than usual, but a surface zone in the lowest quarter remained quite unstained. In the last experiment of the series, the staining of the muscle was examined 7 days after ligature of the lower vessels. By now, the whole muscle appeared normally stained with the exception of a small oval area near the lower extremity where it was less intense (Text-fig. 2*d*). Section of the muscle showed macroscopically a deep coloration of its substance everywhere apart from a very shallow surface zone on its anterior aspect corresponding to the patch of lighter staining already mentioned.



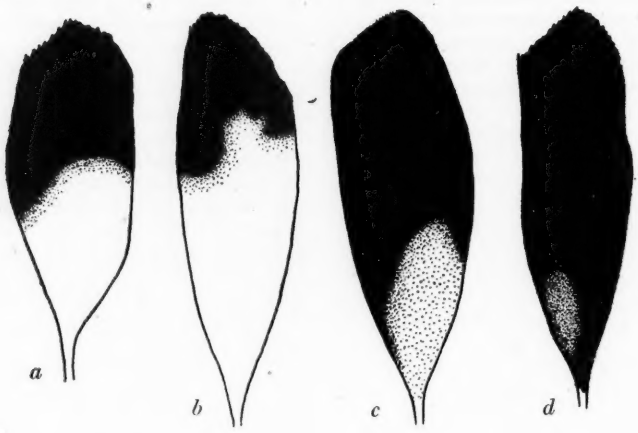
Text-fig. 1. Drawing of a tibialis anterior muscle injected with India ink to demonstrate the vascular pattern. Two vessels from a common trunk enter it near the upper part of the medial border, and from their branches anastomotic loops are formed. A fine vessel extends up the tendon of insertion, and a number of small vessels enter the muscle at its origin. $\times 1\frac{1}{2}$.

These observations appear to indicate that the intramuscular anastomotic connexions in the tibialis anterior are not adequate to permit of a normal blood supply to the muscle for a period of approximately 7 days after interruption of one of its main vessels of supply.

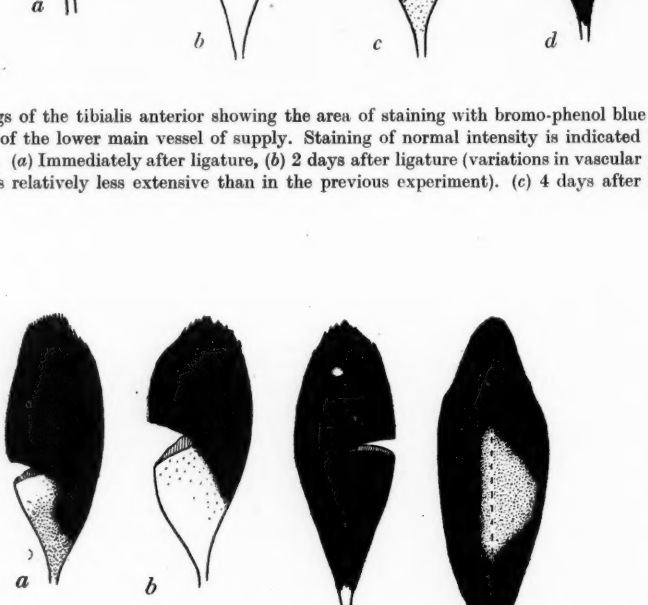
Ligature of both main vessels of supply. In another series of rabbits, both the upper and lower vessels supplying the tibialis anterior were ligatured, so that the only source of blood supply left to the muscle was by way of the fine communicating channels at the site of its origin from the tibia and the slender vessel running up the tendon of insertion. Two days following this operation, blue staining of the muscle is limited rather sharply to approximately its proximal third, but is much less intense here than in normal muscle. It is thus evident that the connexions between the intramuscular vascular plexus and the periosteal vessels at its origin are sufficiently good to allow the rapid diffusion of the dye into the fibres of the proximal third of the muscle at this stage, but the low intensity of the staining indicates that the functional efficiency of the circulation within this part of the muscle is much below its normal level. In its distal two-thirds, the muscle is unstained and is seen macroscopically to contain numerous small haemorrhages.

After 4 days, the blue staining in the proximal part of the muscle reaches its normal intensity but is still limited to about its proximal third. This part of the muscle thus appears now to be adequately vascularized from the periosteal vessels at the site of its origin from the tibia. The distal extremity of the muscle also shows a small zone of faint staining, extending up for a little distance from the tendon, indicating that some degree of vascularization of this part of the muscle has been effected by way of the small artery accompanying the tendon.

After 8 days, blue staining of normal intensity extends downwards through the proximal half of



Text-fig. 2. Drawings of the tibialis anterior showing the area of staining with bromo-phenol blue in four experiments following ligature of the lower main vessel of supply. Staining of normal intensity is indicated in black, and feeble staining in stipple. (*a*) Immediately after ligature, (*b*) 2 days after ligature (variations in vascular pattern explain why the stained area is relatively less extensive than in the previous experiment). (*c*) 4 days after ligature; (*d*) 7 days after ligature.



Text-fig. 3. Drawings of the tibialis anterior showing areas left unstained by bromo-phenol blue after interruption of intra-muscular vessels. (*a*) Hemisection involving medial half of muscle at junction of middle and lower thirds. (*b*) Hemisection involving medial half in the middle of the muscle. (*c*) Hemisection involving lateral half of muscle. (*d*) Median incision down centre of muscle. (*d'*) Transverse section through middle of same muscle showing lack of penetration of bromo-phenol blue into the muscular tissue lateral to the incision.

the muscle, and the distal half is faintly coloured except for a small zone in the lowest quarter which still appears to be quite unstained. The distribution of the staining shows that the functional vascularization of the distal half has been effected from above except for the extremity adjacent to the tendon which is supplied by the artery accompanying the latter.

In an experiment in which the animal was killed 11 days after devascularization the whole muscle was stained with normal intensity except for a sharply defined oval patch over the distal half of the posterior surface which remained completely unstained. A sagittal section showed macroscopically a circumscribed zone of unstained tissue in this region, extending from the posterior surface to the centre of the muscle.

At the end of 14 days, the muscle becomes stained evenly throughout with an intensity which is perhaps rather deeper than usual. It may thus be inferred that by this time the functional revascularization has become complete and that it is almost entirely effected by vessels which reach the muscle through its origin.

In the last experiment of this series, the animal was killed 21 days after ligature of the two main vessels of supply to the tibialis anterior. The muscle was stained to normal intensity with bromo-phenol blue, and was only to be distinguished on superficial inspection from the normal muscle of the opposite (unoperated) side by the fact that the coloration had a slightly purple tinge and was a little smaller in size (weighing 2·0 g. as compared with 2·6 g.).

Interruption of intramuscular vessels. In the series of experiments next to be described, the efficiency of intramuscular anastomotic connexions between branches of the same muscular artery of supply in the tibialis anterior was studied by making incisions to interrupt vascular channels and noting the subsequent distribution of blue coloration following injection of bromo-phenol blue. After hemisection of the muscle transversely through its medial half at the junction of its middle and lower thirds immediately before injection of the dye, a small zone of muscle below the incision remains completely unstained, and below this the medial half of the muscle is only faintly coloured (Text-fig. 3*a*). A reference to the diagram of the vascular pattern in Text-fig. 1 shows that the incision had interrupted the main vessel descending inside the muscle along its medial margin, probably leaving available anastomotic connexions by way of primary branches running down obliquely towards the lateral margin. These connexions, however, are evidently not immediately adequate for the complete vascularization of the distal third of the muscle. In another animal, the hemisection was made on the medial side of the centre of the muscle above the level at which the anastomotic connexions referred to leave the main descending vessel. In this case, the distal half of the muscle remained almost entirely unstained up to a line extending obliquely downwards and laterally from the centre of the muscle to the lateral margin (Text-fig. 3*b*). In contrast with these results, a hemisection through the middle of the muscle involving its lateral half leads to no apparent interference with the blood supply, for the whole muscle stains with normal intensity after injection of bromo-phenol blue (Text-fig. 3*c*).

The effect of a median incision through the central part of the longitudinal axis of the tibialis is shown in Text-fig. 3*d*. In this experiment the animal was injected with bromo-phenol blue and killed 24 hr. after the incision had been made. The tibialis anterior was stained blue normally everywhere except for a zone to the lateral side of the median incision where the surface coloration was below normal intensity. A transverse section through the middle of the muscle showed a central zone in the lateral half which was unstained (Text-fig. 3*d'*). On the other hand, the central area of the medial half was stained more intensely than usual. This experiment demonstrates a limited efficiency of the lateral margin anastomoses of the tibialis anterior. They permit adequate vascularization of the lateral margin itself, and of the surface of the lateral part of the muscle. But they are inadequate to supply the central zone of the lateral part.

We have seen that a considerable part of the tibialis anterior retains some degree of vascularization (so far as this is indicated by rapid staining with bromo-phenol blue) immediately after ligature of both its main vessels of supply, and that this is effected by fine anastomotic connexions with periosteal vessels in its area of attachment to the tibia. The importance of these connexions was further studied by detaching the muscle completely from its origin without interfering with the main vessels of supply, and then injecting the dye. The whole muscle stained with normal intensity (including the detached surface), from which it may be inferred that the connexions are not essential for the adequate vascularization of the muscle in the region of its attachment.

A further experiment was carried out to see whether stripping off the sheath of the muscle has any effect on its vascularization, but, again, the result was negative, for the muscle stained normally after injection of the dye.

THE VASCULARIZATION OF THE SEMIMEMBRANOSUS MUSCLE

The semimembranosus in the rabbit receives its main blood supply by a branch from the profunda femoris artery which enters its deep surface in its proximal third. A second vessel, much smaller, is a branch of one of the sural arteries and enters the deep aspect

of its distal third. The intramuscular vascular pattern, as seen in a preparation from an animal injected with India ink, is shown in Text-fig. 4. Three main branches of the upper vessel of supply descend longitudinally in the muscle, more or less evenly spaced, and are interconnected with each other by anastomotic loops at the lower extremity, and also with branches of the lower vessel of supply.

Text-fig. 4. Drawing of a semimembranosus muscle injected with India ink to show the vascular pattern. An artery (*a*) enters the muscle in its upper third, and a smaller artery (*b*) in its lower third. Some small vessels also enter the muscle at its attachment. $\times 1\frac{1}{2}$.

In a number of experiments the vascularization of the distal third of the semimembranosus was studied after interruption of some of its sources of blood supply.

Complete transection of the muscle at the junction of its middle and lower thirds has no effect on its vascularization so far as this is indicated by the rapidity and intensity of staining with bromo-phenol blue; in other words, the lower vessel of supply is adequate for the distal third of the muscle without the assistance of the descending branches of the upper vessel, and this is found to be the case even if the tendon of insertion is also cut through. On the other hand, ligature of the lower vessel by itself also leads to no apparent effect on the vascularity, and it appears therefore that the distal third of the muscle can be equally well supplied with blood by the descending branches of the upper vessel.

The importance of these descending branches was studied by making partial transections of the semimembranosus at the junction of its middle and lower thirds after preliminary ligature of the lower vessel of supply. In two rabbits the anterior half of the muscle was transected almost to the median axis of the muscle, in order to interrupt at least one of the descending branches. In one animal, which was injected with bromo-phenol blue and killed 24 hr. after the operation, the muscle was everywhere stained below the level of the incision but, except at its distal extremity close to its insertion, the coloration in its anterior half was less intense than elsewhere. Transverse sections of the distal third showed macroscopically a normal staining in the substance of the muscle except for a small zone near its anterior margin below the incision. The second animal was injected and killed 4 days after the operation, and by this time the distal third of the muscle appeared to be completely vascularized, for the staining with bromo-phenol blue was of normal intensity. Thus it appears that the vascularization of the distal third of the semimembranosus can certainly be effected, with some temporary impairment, by two of the descending branches of the upper main vessel of supply.

In another experiment, approximately two-thirds of the width of the muscle were transected at the junction of its middle and lower thirds, thereby interrupting at least two of the descending vessels, and the animal was injected and killed 24 hr. later. In this case the interruption of the blood supply to the distal third of the muscle was much more severe, for, as seen in Text-fig. 5*a*, a considerable part of the muscle below the level of the incision remained quite unstained. Section of the muscle in this region showed macroscopically that the muscle substance in its anterior half was unstained except for a narrow marginal zone, and that the boundary between the stained and unstained areas was quite sharply defined.

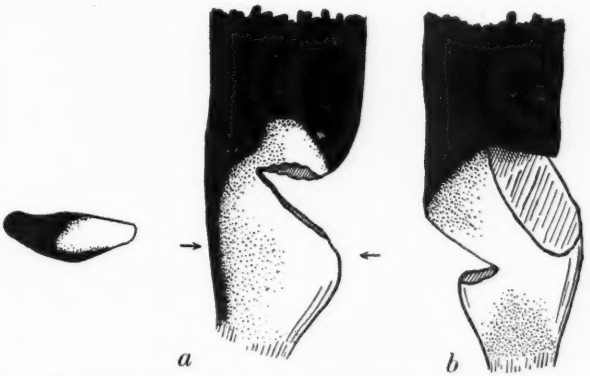
Lastly, the effect was studied of a double hemisection of the semimembranosus at different levels in its lower third, the effect of which is to ensure the interruption of all the main descending branches, leaving available as the only possible routes of blood supply the secondary intercommunications between these branches, as well as small vessels entering the lower extremity of the muscle through its insertion. As seen in Text-fig. 5*b*, the muscle below the incision

remained almost entirely unstained 24 hr. later except for a slight coloration extending up from its insertion.

In the next series of experiments to be described, the distal third of the semimembranosus was separated from the proximal two-thirds by a complete transection and the lower vessel of supply ligatured. The intention was to see how far the distal third of the muscle could be effectively revascularized through communicating vessels reaching it by way of its insertion. The attachment of the distal end of semimembranosus is by a membranous expansion, and thus contrasts with the broad fleshy attachment of the tibialis anterior at its origin.

THE VASCULARIZATION OF THE GRACILIS MUSCLE

In the rabbit the gracilis is a broad thin sheet of muscle, vascularized on its deep aspect from several sources. There are two main vessels of supply, one direct from the femoral artery and one from the obturator. These vessels (*a* and *b* in Text-fig. 6) descend to form a broad anastomotic loop in the distal third of the muscle and give off numerous branches. In addition, a variable number of smaller vessels enter the proximal third after piercing the subjacent adductor muscles, and their branches effect anastomoses with those of the main vessels of supply.



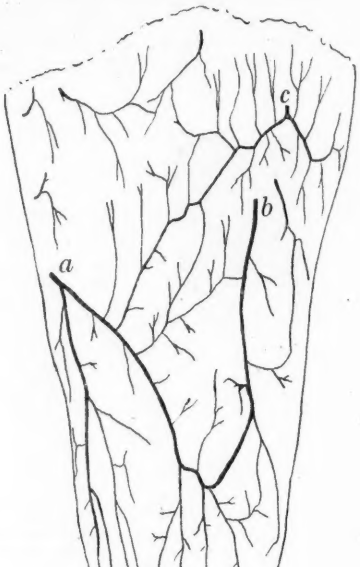
Text-fig. 5. Drawings of the lower third of semimembranosus muscle showing the effect of incisions on its vascularity. (*a*) Two-thirds of the muscle had been cut through 24 hr. previously, and as a result a considerable area below the level of the incision remained unstained after injection of bromo-phenol blue. The arrows indicate the level of the transverse section which is shown to the left. (*b*) Showing the effect of double hemisection of the muscle at different levels 24 hr. previously.

Two days following this operation, the distal third of the semimembranosus remains unstained after injection with bromo-phenol blue, except for a faint coloration close to its insertion. After 4 days the blue staining at the insertion end was found to be quite intense, extending up into the muscle for about 10 mm. Above this level there is a patchy distribution of faint coloration in the substance of the muscle, but most of the latter remains unstained. In a third animal, injected and killed 8 days after operation, the whole of the distal third of the muscle had acquired a faint blue coloration except for a rather well-circumscribed central core which still remained unstained. At the end of 14 days, the muscle, though considerably shrunken, stained with normal intensity.

Lastly, it may be noted that detachment of the semimembranosus from its insertion without interrupting any of the main vessels of supply is without any effect on the rapid normal staining of the muscle with bromo-phenol blue.

Ligature of the main vessels of supply to the gracilis, whether separately or together, has remarkably little effect on the vascularity of the muscle as judged by the diffusion of bromo-phenol blue. Immediately after ligature of the branch from the femoral artery, the area to which it is mainly distributed is slightly less deeply coloured than the rest of the muscle, but there is no sharp line of demarcation. In an experiment in which the main branch from the obturator artery was ligatured and the dye injected immediately afterwards, the muscle stained everywhere with normal intensity, but in this particular animal the other main vessel of supply (from the femoral artery) was found to be relatively larger than usual. In another experiment the main branch from the obturator was ligatured 24 hr. before injection of the dye. The muscle was everywhere stained, but less intensely than usual in its lower three-quarters. In this animal the vessel of supply from the femoral artery was found to be considerably smaller than usual. In one rabbit, both

the main vessels of supply were ligatured and, again, the whole muscle was stained (but less intensely than usual in its lower two-thirds) when, 24 hr. later, the bromo-phenol blue was injected. It was evident that in this case the muscle was able to be well vascularized by the small additional vessels which enter the proximal third of the muscle above the main vessels of supply. The effect was then studied of ligaturing all the main and subsidiary vessels entering the deep aspect of the gracilis and detaching



Text-fig. 6. Drawing of a gracilis muscle injected with India ink to show the vascular pattern. A branch of the femoral artery (*a*) enters the muscle near its lateral border, and a branch of the obturator artery (*b*) near the upper third of its medial border. In the upper third of the muscle a variable number of small branches of the obturator artery (*c*) also supply the muscle. $\times \frac{4}{5}$.

the muscle from its insertion, thus leaving intact only the fine communicating vessels which reach it through its attachment at its origin. The animal was injected and killed 24 hr. later, when it was found that, while most of the proximal third of the muscle had taken up the blue dye to a normal intensity, the distal two-thirds were quite unstained, and there was a sharp line of demarcation between the two zones.

Lastly, in a series of acute experiments, individual branches of the main vessels of supply were ligatured to see whether localized areas of devascularization could be induced by this method. The gracilis was detached from its insertion and held up so that the branches could easily be seen in the translucent sheet of muscle, and they were then ligatured individually by means of a threaded needle. In no case did these operations lead to any defect in staining with bromo-phenol blue.

THE CIRCULATORY CONDITIONS IN THE 'DEVASCULARIZED' MUSCLE TISSUE

The fact that a portion of a muscle remains unstained 4 min. after the injection of bromo-phenol blue is completed cannot of course be taken as evidence that there is no circulation at all here. Rous & Gilding (1929) demonstrated that there was not complete cessation of interchange between the blood and tissue where in their experiments bromo-phenol blue failed to penetrate. They found, for example, that Patent Blue V (whose diffusibility is approximately twice as great) may slowly colour an area left unstained by bromo-phenol blue. On the other hand, they quote the earlier observations of Rous & Drury (1929) that in skin areas which bromo-phenol blue failed to penetrate an acidosis develops, due to the local accumulation of acid metabolites, and conclude that to all intents and purposes the circulation in these areas had ceased to be effective. A circulation may thus be present, but its functional efficiency may be too low to permit the diffusion of the dye from the capillaries into the muscular tissue within such a short space of time. This factor was studied by watching the diffusion of the dye in partially devascularized muscles of rabbits under continuous nembutal anaesthesia. After exposure of the muscles (which were kept constantly moistened with saline) the dye was injected and its diffusion observed for 2 hr. subsequently. At the end of this time, the dye begins to fade in the muscles generally, so that the limits of staining become difficult to define.

In a tibialis anterior muscle of which the lower vessels of supply were ligatured immediately before the injection of the dye, approximately the upper half of the muscle became stained within 2 min. At the end of half an hour, the blue area had extended distally for 18 mm. in the lateral half of the muscle and to a lesser extent in the medial half. At the end of 1 hr., the coloration had spread over most of the muscle, except for an area on the medial side of its distal half which still remained unstained. Half an hour later, this area was faintly coloured and no further change was observed at the end of 2 hr. When the muscle was removed after exsanguination, fixed and sectioned longitudinally, the distal half of the muscle appeared almost unstained, in marked contrast with the blue colour of the proximal half. It seemed probable, therefore, that the coloration of the distal half observed during life was caused by a feeble circula-

tion carrying the dye through the main blood vessels, but inadequate to permit its ready passage through the capillary bed and its diffusion into the muscular tissue. This inference was confirmed in another case in which the tibialis anterior was inspected immediately after ligature of both its main vessels of supply and during the injection of the bromo-phenol blue, for the dye was seen to enter a superficial vein in the distal part, although the muscular tissue itself remained unstained.

In a further experiment, the tibialis anterior muscle was observed in the same way 24 hr. after its lower vessels of supply had been ligatured. A few minutes after injection of the dye, approximately the upper third of the muscle became stained, with a sharp line of demarcation from the distal unstained portion. Half an hour later the appearance was unchanged. After 1 hr., diffuse and patchy blue staining of faint intensity appeared over the distal half of the muscle, and this appearance remained stationary up to the end of 2 hr. Longitudinal section of the muscle after removal showed staining of the proximal third. In the distal two-thirds most of the muscular tissue was unstained, but round a dilated vein running obliquely down the centre of the muscle was a perivascular zone of fairly deep coloration which had resulted from the slow diffusion of the dye into the surrounding tissue.

These observations indicate the existence of a circulation in the 'devascularized' portions of the muscles sufficient to allow the passage of the dye through anastomotic communications. After 2 hr., however, the greater part of the 'devascularized' muscular tissue still remained unstained, and from this it may be inferred that the adjustment of the circulation by way of these communications is certainly not adequate to permit of an efficient capillary circulation through the tissue.

The circulation in the 'devascularized' portion of the tibialis anterior was further investigated by ligature of the lower vessels of supply immediately *after* injection of the bromo-phenol blue, in order to see to what extent the removal of the dye is prevented or retarded. In one experiment, the rabbit was killed 6 hr. later, by which time the muscles generally had regained their normal colour. The lower two-thirds of the tibialis anterior still retained a very faint tinge of blue as seen on surface inspection, but the substance of the muscle exposed in section appeared to be quite unstained. In a second experiment, the animal was killed 24 hr. after injection of the dye followed by partial devascularization. In this case, the tibialis anterior showed a faint bluish tinge on the surface over its lower two-thirds, and a section of the muscle showed that its substance was still distinctly stained, though much more faintly than in a normal muscle a few minutes after injection of the dye. It thus appears from these two experiments that a few hours after ligature of the lower vessels of supply of the tibialis anterior, a circulation of some kind exists in the affected part of the muscle through which most of the bromo-phenol blue is removed, but it is not adequate for its complete removal at least up to 24 hr.

The relation of the areas of muscle unstained by bromo-phenol blue to the circulatory pattern was also studied in the following experiment. A rabbit, in which the lower vessels of supply to the tibialis anterior had been ligatured 2 days previously, was injected with bromo-phenol blue, and then, under nembutal anaesthesia, India ink diluted in normal saline was introduced with a hypodermic needle directly into the abdominal aorta so that it was carried into the hind-limbs with the circulating blood. Approximately 10 c.c. were allowed to flow into the circulation in this way, and the aorta and inferior vena cava then immediately clamped. After fixation, the tibialis anterior was removed and cleared in oil of wintergreen. The muscle was normally stained with the blue dye in its proximal half, and the distal half was faintly coloured but showed small patches in which the muscle tissue was completely unstained. Examination of the specimen under a binocular microscope with transmitted light showed that the India ink had filled the finer blood vessels to a density approximately corresponding to the density of the blue staining. The patches of unstained muscle show no injection of the capillary bed, though they are in some cases traversed by main vessels containing India ink. There seems no doubt, therefore, that the absence of staining with bromo-phenol blue after the ligature of the vessels of supply to a muscle can be taken to indicate a gross interference with its normal vascularization, at least in so far as the capillary circulation is concerned. The effect of this on the muscular tissue itself is clearly shown by histological studies, the details of which are described below. From these it will be seen that all areas where the muscle tissue had failed to stain were found to contain muscle fibres which had undergone marked degenerative changes, and that at least the majority of these fibres undergo subsequent absorption.

HISTOLOGICAL CHANGES ASSOCIATED WITH DEVASCULARIZATION OF THE TIBIALIS ANTERIOR MUSCLE

Two days after ligature of the two main vessels of supply of the tibialis anterior, the following picture is seen in longitudinal sections of the muscle which have been stained with haematoxylin and eosin. In the proximal third (corresponding in extent almost exactly with the region which had become stained after injection of bromo-phenol blue) the muscle appears normal except for the presence of dilated blood vessels. In the distal two-thirds there is a considerable cellular infiltration extending between

the individual muscle fibres, associated with small scattered haemorrhages and oedema of the endomysial tissue. The cellular exudate consists mainly of large numbers of polymorphonuclear leucocytes and a small proportion of macrophages. The muscle fibres show evident signs of degenerative change (Pl. 2, fig. 4). They are swollen and stain weakly with eosin, and over considerable parts of their length are apparently devoid of nuclei. Thus they contrast strongly with the fibres in the proximal third of the muscle. On the other hand, they preserve their cross-striations, and for the most part retain an even contour, though most of them are broken into short segments. Here and there, however, muscle fibres are seen in process of complete dissolution, fragmented, irregular in outline, vacuolated, erratically staining, showing a complete absence of cross-striation, and sometimes filled with a coarse basophil granular deposit (Pl. 4, fig. 17). These necrotic fibres are particularly evident at the junctional zone between the proximal third and distal two-thirds of the muscle, alongside a dilated main vessel which extends down the centre, at the surface of the muscle, and near its lower extremity. In these regions, the cellular infiltration among the muscle fibres is denser than elsewhere. At the lower extremity close to the tendon of insertion muscle fibres of normal healthy appearance are present.

After 4 days the histological picture is very similar (Pl. 1, fig. 1). In the proximal third of the muscle the vascular reaction is now more marked, and the dilated vessels have become surrounded by newly formed fibroblastic tissue. In the distal two-thirds, the cellular infiltration among the muscle fibres is less intense, and is composed of a larger proportion of macrophages, many of which contain coarse basophil granules. There is considerable congestion of the vessels in this part of the muscle, especially the main venous channels which are distended with blood. The junctional zone of necrosis between the proximal and middle thirds of the muscle is conspicuous and necrotic fibres are also found in the immediate neighbourhood of main blood vessels extending through its central axis, particularly towards the lower extremity. The muscle fibres in the distal two-thirds of the muscle are almost all in an advanced state of degeneration with complete absence of nuclei. Nuclei are still present, however, in scattered fibres close to the surface of the muscle, and alongside the dilated vessels extending down its central axis.

Sections of a tibialis anterior muscle removed 5 days after ligature of both vessels of supply show evident signs of recuperation. The contrast between the proximal and distal parts of the muscle is in general similar to that already noted above, but the cellular infiltration of the distal two-thirds and the oedema of the endomysial tissue are much diminished. In the exudate, polymorphonuclear leucocytes are now relatively few, while macrophages predominate more. In all the zones where previously necrosis of muscle fibres was observed, there is now a richly cellular tissue of a fibroblastic nature. In this tissue strands of nucleated protoplasm run longitudinally, having all the appearance of myoblasts (Pl. 2, figs, 6, 7). The nuclei are frequently arranged in a closely packed row, resembling the 'myotube' stage of myogenesis, and strands are occasionally to be seen in continuity with the ends of healthy muscle fibres adjacent to the zone. Elsewhere the 'stumps' of surviving muscle fibres give the appearance of sending out richly nucleated buds of protoplasm into the fibroblastic tissue, and it seems probable that the myoblastic elements are all derived eventually from these buds. Thus it is clear that active regeneration of muscle fibres is taking place into the zones from which necrotic portions of fibres have been previously removed. In these areas portions of completely necrotic fibres are still present, apparently in process of removal by the activity of macrophages. Lastly, in the central axis of the muscle and at its posterior surface are a few nucleated muscle fibres of normal appearance which, it seems, have resulted from the recovery of some of the fibres which in the earlier stages had shown degenerative changes (Pl. 3, fig. 12).

After 8 days, regeneration of muscle tissue has proceeded further. In the zones already referred to, reconstituted muscle fibres now appear as long narrow strands staining well with eosin and containing abundant nuclei. The latter are disposed in characteristic clumps ranged down the centre of the strands, the protoplasm is longitudinally fibrillated, and here and there a faint cross-striation is visible (Pl. 2, fig. 8). In the distal half of the muscle there are still many old fibres showing the degenerative changes already described, i.e. loss of nuclei, swelling of the contractile substance, and weak staining with eosin. The cellular exudate in this part of the muscle is further reduced, and consists now almost entirely of macrophages.

After 11 days, most of the distal half of the muscle (as seen in a median section) is composed of fibroblastic tissue filled with actively regenerating muscle fibres (Pl. 1, fig. 2). Old fibres showing characteristic degenerative changes occur in two well-defined areas, one in the posterior part of the distal half of the muscle (corresponding to the unstained area noted after injection with bromo-phenol blue) and a smaller area in the centre of the muscle. In these areas the fibres still preserve their cross-striation, but they seem to be in process of absorption as they become encroached upon by the surrounding fibroblastic tissue, and many contain macrophages within their substance. At the same time some of them give the appearance of sending out richly nucleated strands of protoplasm into the fibroblastic tissue

which are apparently giving rise to myoblastic elements. At the periphery of the areas occasional normal fibres are seen, suggesting that some of the old fibres have recovered from their earlier degenerative changes. At the lower extremity of the muscle, close to the tendon of insertion, many of the original muscle fibres have survived intact, showing normal staining and normally disposed nuclei.

In sections of the muscle which had been devascularized 14 days before the animal was killed, the newly formed muscle fibres extend throughout the length of the previously necrotic zones, and are particularly abundant in the middle third of the muscle (Pl. 1, fig. 3). They show a more distinct cross-striation, and the nuclei are more evenly spaced along their length and partly displaced to a peripheral position (Pl. 2, fig. 9). The fibres have also increased in thickness, though most of them are still much finer than the normal fibres of the tibialis anterior muscle. The cellular infiltration among the muscle fibres of the distal part of the muscle has disappeared. In the central axis of the muscle there are many fibres of almost normal appearance which are probably surviving elements of the original muscular tissue.

In a tibialis anterior muscle which had been devascularized for 3 weeks, the histological picture is similar to the previous specimen in that the degenerated muscular tissue has been entirely removed and replaced by young regenerated fibres. The latter are for the most part fine in calibre, but they have distinct cross-striations and many of their nuclei are displaced to a peripheral position. They extend through the length of the muscle which intervenes between regions of normal muscular tissue which had remained vascularized, that is to say, in the proximal parts of the muscle and at the lower extremity. In the latter region, normal muscular tissue seems more abundant than in the other experiments in which both the main vessels supplying the tibialis anterior had been ligatured, presumably related to the fact that the blood supply by way of vessels extending up the tendon of insertion was more effective than usual. At this stage, however, it is difficult at junctional zones to distinguish between those muscle fibres which have regenerated and those which are old surviving fibres. Many of the fibres near the lower extremity of the muscle appear approximately normal in size, but they are much more richly nucleated than normal fibres. Further, the nuclei are often central in position, and are more rounded and less elongated than in mature tissue. Transverse sections through the junction of the middle and lower thirds of the muscle show that in this region it is composed almost entirely of young regenerated fibres (Pl. 3, fig. 11). Surviving old fibres persist in a narrow zone at the surface, here and there alongside main vessels in the centre, and in a small compact group at one side of the muscle. The regenerated fibres are circular in transverse section, and are separated from the walls of their endomysial tubes by a relatively wide space. Herein they contrast strongly with transverse sections prepared through the corresponding region of the tibialis anterior of the opposite (unoperated) side, for here the fibres have the typical polygonal shape resulting from mutual pressure.

It may be noted that, in this 3 weeks' experiment, bromo-phenol blue was injected into the animal immediately after the main vessels of supply had been ligatured in order to ascertain the extent of immediate devascularization. The whole of the distal half of the muscle and the medial part of the proximal half remained completely unstained. Thus it is apparent that the devascularization was at least as extensive as in those experiments in which, after 2 and 4 days, the fibres of the distal half of the muscle were found to be almost completely degenerated. It may be inferred, therefore, that in the present experiment, the degenerated distal part of the muscle had been largely reconstituted by the regeneration of new muscular tissue.

The histological changes in the devascularized tibialis anterior may be summarized as follows. Within 2 days following ligature of the two main vessels of supply, the proximal third retains its normal structure but, with a sharp line of demarcation, the distal two-thirds have undergone extensive degeneration. Some fibres survive at the lower extremity of the muscle (where they evidently obtain some blood supply from fine vessels extending up the tendon of insertion), in the central axis alongside main vascular channels through which a feeble circulation is maintained by anastomotic connexions, and at the surface of the muscle where they have a close relationship to epimysial tissues (Pl. 3, fig. 13). In the same regions, and also at the junctional region between the proximal third and the distal two-thirds of the muscle, are zones of active necrosis where muscle fibres rapidly undergo complete disintegration. The bulk of the muscle tissue in the distal two-thirds undergoes a passive autolysis in the process of which the muscle fibres become swollen, lose their nuclei, break up into short segments and stain weakly with eosin, but preserve their cross-striations. The necrotic zones are rapidly infiltrated with fibroblastic tissue, and in the course of a few days this tissue invades the rest of the degenerated muscle, removing it systematically by phagocytic action. Active regeneration proceeds concurrently with this process from myoblastic elements which are derived partly from muscle tissue which retains some degree of vascularization at the proximal and distal ends of the muscle, and partly from old surviving muscle fibres which persist at the surface and in the immediate neighbourhood of main vessels in the substance of the muscle. In the course of 2 weeks the degenerated muscular

tissue has all been removed and has been replaced by newly formed muscle fibres to such an extent that the whole muscle is evidently in process of being largely reconstituted (cf. figs. 1, 2 and 3 in Pl. 1).

The histological changes in the tibialis anterior of which the lower vessels of supply only had been ligatured are comparable with those already described, except that as a rule less of the distal part of the muscle is affected by degenerative changes. In one case, 2 days after the operation, actual necrosis is confined to the lower extremity of the muscle in the neighbourhood of the tendon of insertion. Extending up from this localized area is a zone of cellular infiltration in the endomysial tissue, mainly at the surface of the muscle, but the muscle fibres themselves appear unaffected. The proximal half of the muscle is quite normal. In another experiment with the same time interval, the distal half of the muscle is more profoundly affected, perhaps related to the fact that in this case some variation in the vascular pattern had led to a greater disturbance of the blood supply as the result of ligature of one of the main vessels. While the proximal third of the muscle is normal, in the distal two-thirds there is an extensive cellular infiltration and the area is scattered with segments of fibres undergoing necrosis. The capillaries are all very congested, being filled with rows of closely packed corpuscles.

After 4 days of partial devascularization the muscle is almost normal except for its lowest quarter. Here, there is still a great degree of cellular infiltration and some necrosis of muscle fibres. On the anterior surface of this part of the muscle regeneration is in progress, for richly nucleated protoplasmic strands are seen extending into a zone of loose fibroblastic tissue from the ends of normal fibres, and here and there typical myotubes are present.

Lastly, in a muscle whose lower vessel of supply had been ligatured 7 days previously, the muscle is normal except for a surface layer on the anterior aspect of the distal half. Here, there is a zone of loose fibroblastic tissue containing spaced-out regenerated muscle fibres of fine calibre, with faint cross-striations and richly nucleated. Many of the nuclei are disposed in tight clumps of six or seven. A circumscribed area of degenerated muscle fibres still persists in the same region.

From these observations it is apparent that ligature of the lower vessel of supply to the tibialis anterior is usually much less destructive in its effects on the muscular tissue than ligature of both vessels. An effective collateral circulation to the distal half of the muscle is fairly rapidly established so that most of the muscle fibres in this region survive without undergoing complete degeneration. Nevertheless, in all the experiments zones of fibres undergoing hyaline necrosis are found towards the distal end of the muscle, which become rapidly replaced by newly regenerated fibres.

HISTOLOGICAL CHANGES ASSOCIATED WITH THE DEVASCULARIZATION OF THE LOWER THIRD OF THE SEMIMEMBRANOSUS MUSCLE

As already described, in a series of experiments the distal third of the semimembranosus was deprived of all blood supply except such as might be able to reach the muscle through its membranous insertion. Two days after this operation, the whole of the muscle segment is extensively infiltrated with leucocytes. In its central portion the muscle fibres preserve their cross-striation, but in many places they are devoid of nuclei. At the cut end of the muscle, at its lower extremity, and in narrow surface zones, most of the fibres are in process of active necrosis, undergoing fragmentation and vacuolation, and their cross-striations have disappeared. After 4 days, there is a conspicuous invasion of the lower extremity by dense granulation tissue, carrying with it a leash of dilated blood vessels which extend up the centre of the muscle. The granulation tissue contains clumps of darkly stained necrotic tissue, and necrotic muscle fibres are also found close to the surface of the muscle, at its cut end, and in the neighbourhood of the dilated vessels. Elsewhere the fibres preserve their continuity and cross-striations, but they are swollen and show almost everywhere a complete absence of nuclei. At the surface of the muscle and alongside the central dilated vessels are scattered fibres in which the nuclei persist intact. The interfibrillar cellular infiltration remains conspicuous.

The material from the experiment in which the distal end of the semimenbranosus was isolated except for its insertion 5 days previously shows evidence of rapid recuperation. While the muscle tissue close to the insertion appears normal, the bulk of the muscle is made up of fibres in which nuclei are absent and which are separated from each other by a cellular exudate and endomysial oedema. The remains of necrotic fibres are present at the cut extremity and at the surface of the muscle, but these zones have now become filled with fibroblastic tissue containing fine strands of eosinophil protoplasm with nuclei frequently disposed in rows. These are evidently new muscle fibres in process of regeneration (Pl. 2, fig. 5, and Pl. 3, fig. 14).

In the 8-day specimen, the histological picture is much the same, except that in the surface zones the regeneration is more advanced, for the newly formed muscle fibres have in some places increased in thickness and acquired a longitudinal striation. Their nuclei are more evenly disposed throughout their length (though here and there still arranged in

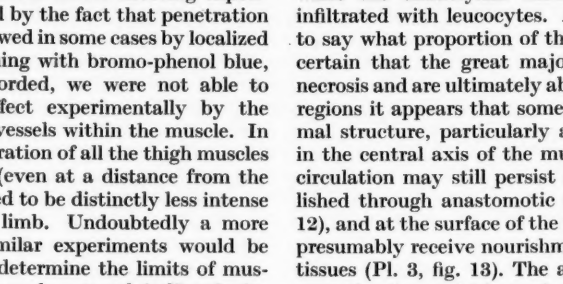
clumps), and many of them occupy a peripheral position. In this case, also, there is a small central core of necrotic fibres which was not present in other experiments, suggesting that the revascularization of this region has been delayed too long.

In the 14-day specimen there has been a considerable amount of muscle destruction and subsequent replacement, for the bulk of the muscle segment is composed of very numerous fine fibres in the process of regeneration and is permeated with abundant vascular granulation tissue.

THE EFFECT OF GUN-SHOT WOUNDS ON THE VASCULARITY OF MUSCLE

Eight experiments on rabbits were carried out in which a steel bearing of $\frac{5}{32}$ in. diameter was shot through the thigh at velocities varying from 250 to 1000 m. per sec. The animals were injected with bromo-phenol blue and killed after times varying from a few minutes to 4 days. Since it was not possible to place the shot accurately with regard to individual muscles and their blood supply, the resultant vascular defects, as indicated by the distribution of staining in the muscles, varied considerably. The shot made a clear puncture wound of 4–5 mm. in diameter in the muscles which it penetrated, and in all cases the muscular tissue immediately surrounding the track was unstained by the bromo-phenol blue to a distance of 2–3 mm. In some cases, however, larger areas showing absence or weakness of staining were produced in the neighbourhood of the missile track. Thus among our records are a wound involving the lower part of the semimembranosus in which, 24 hr. later, the centre of the lower third of the muscle appeared quite unstained, a penetrating wound of the adductor magnus associated 24 hr. later with a zone of vascular impairment extending upwards in the muscle for 18 mm., and a wound through the gracilis which produced a zone of devascularization extending over an area of 14 by 8 mm. These effects are evidently due to a vascular involvement, for they are too extensive to be ascribed to the direct traumatic injury of the muscular tissue by the missile. This is also shown by the fact that the areas of devascularization are usually quite asymmetrical with regard to the missile wound, often being located mainly to one side of it, and by the variability of the areas in different muscles and different animals. Thus in one experiment, penetration of the adductor magnus led to a devascularization of muscle tissue limited to the immediate periphery of the missile track, while in another it produced an extensive vascular defect in the muscle. This last case will be described in some detail as an example of what may occur in a gun-shot wound of muscle. Sixty hours after the rabbit had been shot through the adductor region of the thigh, it was injected with bromo-phenol blue and killed. The shot was found to have penetrated the adductor magnus in the centre of its distal two-thirds, forming a small aseptic puncture wound. The muscle showed a clear-cut rectangular area of quite unstained tissue, 24 by 14 mm., in the caudal half of its central third, the penetration wound being situated at the margin of the rectangle (Pl. 4, fig. 15). There was a sharp line of demarcation between the unstained area of muscle and the surrounding area of bright blue coloration. Elsewhere in the adductor magnus there were patches of slight bruising, especially near the distal end at its insertion. The histological picture seen in longitudinal sections of the muscle is quite similar to that already described in the tibialis anterior 2 or 4 days after ligature of its main vessels of supply (Pl. 4, fig. 16). In the devascularized area the muscle fibres are swollen and retain their cross-striations, but they stain weakly with eosin and are devoid of nuclei over considerable parts of their length. They are separated from each other by an endomysial oedema, and there is an extensive interfibrillar exudate of polymorphonuclear leucocytes and macrophages. The vascular channels in this part of the muscle are also congested, many of the capillaries being packed with red corpuscles. The stained portion of the adductor magnus has a normal histological appearance. At the junction of the normal and devascularized areas is a narrow boundary zone of active necrosis of muscle fibres, and a similar zone occurs at the surface of the devascularized region. Here the fibres are completely disintegrated and, since their fragments stain darkly with haematoxylin, the zones are quite conspicuously outlined at a low-power magnification (see Pl. 4, fig. 16). Thus the histological changes in the affected part of the muscle conform with those previously found in experimental devascularization by ligature, and it may be inferred, therefore, that the missile had interrupted the vascular supply to the area. From another animal a preparation of the adductor magnus was made after injection of India ink to demonstrate the vascular pattern in the muscle. As seen in Text-fig. 7, it has two sources of blood supply: the upper comes from the profunda femoris and the lower directly from the femoral artery. It will be noted also that the lower vessel supplies a territory closely corresponding in size and shape to the area of devascularization produced in the experiment, and on the diagram the relative position of the missile track has been plotted to indicate that interruption of the vessel would provide an explanation of the vascular impairment observed. It may be that this is not the whole explanation, for in two rabbits in which we ligatured or cut the lower vessel of supply we did not produce a devascularized area of a size and shape corresponding to that of the shooting experiment, though in both cases a small unstained area was left in the same region after injection with bromo-phenol blue.

In the shooting experiment the intramuscular anastomoses may have been less adequate than usual, or it is possible that the trauma may have induced a local vasospasm affecting the branches of the vessel which had been interrupted and so amplifying the direct effects of the interruption. That vasospasm is a contributory factor in the production of vascular defects in the shooting experiments is also suggested by the fact that penetration of the gracilis was followed in some cases by localized patches of feeble staining with bromo-phenol blue, while, as already recorded, we were not able to produce the same effect experimentally by the ligature of individual vessels within the muscle. In one case, also, the coloration of all the thigh muscles of the wounded limb (even at a distance from the missile track) was noted to be distinctly less intense than in the opposite limb. Undoubtedly a more extended series of similar experiments would be necessary in order to determine the limits of muscular damage which can be caused indirectly by vascular involvement from gun-shot wounds, but our short series demonstrates that these may lead to fairly sharply defined areas of functional devascularization corresponding in extent to the territories of supply of individual vessels. On the basis of our ligature experiments, also, it may be inferred that the circulatory deficiency is likely to last for several days.



Text-fig. 7. Drawing of the lower half of the adductor magnus injected with India ink to show the vascular pattern. The relative position of the missile track in the shooting experiment has been marked in order to demonstrate that the devascularized area in the muscle corresponds with the territory of the artery of supply (*a*) from the femoral artery. × ⅔.

DISCUSSION

Our experiments have demonstrated that complete or partial interruption of the main channels of blood supply to a rabbit's muscle may lead to an effective devascularization of a considerable part of it, lasting for some time. Thus, if the two main vessels to the tibialis anterior are ligatured, complete revascularization is delayed for almost a fortnight, while if one vessel only is ligatured, this may be effected in a week. These conclusions are based primarily on the rapidity and intensity with which bromo-phenol blue, injected into the circulation, stains the muscle. That this method is valid for assessing the circulatory efficiency in muscle has been confirmed by a number of observations, and particularly by the histological study of the experimental material. In muscle tissue which remains uncoloured by the dye when injected a few days after interruption of vessels of supply, the fibres show marked degenerative changes such as disappearance of the nuclei, swelling of the contractile substance and weakness of staining, while the endomysial tissue is oedematous and infiltrated with leucocytes. Although it is difficult to say what proportion of these fibres survive, it is certain that the great majority of them undergo necrosis and are ultimately absorbed. But in certain regions it appears that some can recover their normal structure, particularly alongside main vessels in the central axis of the muscle in which a feeble circulation may still persist or is quickly re-established through anastomotic connexions (Pl. 3, fig. 12), and at the surface of the muscle where they can presumably receive nourishment through epimysial tissues (Pl. 3, fig. 13). The amount of the original muscular tissue which survives alongside the central vessels seems to vary considerably in different animals. Of those fibres that become ultimately absorbed, some undergo rapid fragmentation and form conspicuous necrotic zones in the muscle. These zones occur at the junction of devascularized and normally vascularized muscle, in a narrow surface layer, in the region close to the tendon of insertion, and in the immediate neighbourhood of dilated vessels in the substance of the muscle. Other degenerated fibres, which are at first farther removed from circulating blood, undergo a passive autolysis and are more slowly and systematically removed by the gradual encroachment of invading masses of the fibroblastic tissue which replaces the earlier necrotic zones. With the removal of old damaged muscle fibres the regions which they occupied become rapidly filled with regenerating muscle fibres.

The regeneration of muscular tissue in our preparations is a very striking phenomenon. That the histological appearances are due to a real process of regeneration, and not simply the result of various stages of dedifferentiation of old fibres, is demonstrated by the time sequence of events. The process

occurs first in those regions which are affected by an initial necrosis of old fibres (in the course of which these become fragmented and completely disintegrated) and shows successive phases of development from fine protoplasmic strands of myoblastic tissue to the completely reconstituted fibres. These phases of muscle regeneration have been recorded by previous observers (see v. Meyenburg, 1929), and have been described in recent years by Forbus (1926). The process appears to be identical with that which occurs following hyaline degeneration of muscle in typhoid or pneumonia. According to Forbus complete regeneration takes place in this condition, nuclear proliferation and increase in size of the new muscle elements continuing until a sufficient length is obtained to bridge the gap in the old fibres which resulted from the degeneration. He also notes that the absence of any scar formation or even fibroblastic proliferation after 15 to 20 days of disease is striking, especially when compared with the extraordinary changes in the muscle itself. This description corresponds well with our own observations on devascularized muscle, for the fibroblastic tissue which first infiltrates the necrotic areas becomes rapidly replaced by the development of new fibres so that the muscle appears to be in process of becoming completely reconstituted. Forbus describes similar phenomena in muscles which have been experimentally damaged with such agents as alcohol and phenol, and his description and illustrations of the degenerative and regenerative changes which ensue also coincide with those seen in our material.*

Another striking phenomenon of the regeneration of devascularized muscle is the rapidity with which it occurs, for after a fortnight the muscle appears in sections stained with haematoxylin and eosin to be regaining its normal structure except that in the affected areas the muscle fibres are of smaller calibre than elsewhere, and immature in their structure. However, the time sequence corresponds well with the tabulation of phases of regeneration recorded by Durante (1902) on the basis of observations by various investigators. The earliest times at which these phases occur are given as follows: nuclear proliferation 4 hr., spindle muscle cells (myoblasts) 3 days, young fibres 6 days, striation of young fibres within 3 weeks.

We shall not concern ourselves here with the further histogenetic details of muscle regeneration observed in our preparations. It is hoped later to pursue this enquiry on a more extended basis and with more material. It may be noted, however, that the regenerated fibres are apparently derived from two sources. In the tibialis anterior, after interruption of the main vessels of supply, many seem to be formed by a streaming of nucleated strands of protoplasm from the stumps of normal fibres in the proximal part which is still effectively vascularized through the origin of the muscle, or from normal fibres which persist at the lower extremity of the muscle where their vitality is evidently maintained by the fine blood vessels extending up the tendon of insertion. Myoblastic elements also make their appearance in close relation to the stumps of the degenerated fibres in the devascularized part of the muscle while they are undergoing absorption, and appear to be derived from disintegrated fragments of damaged fibres which, because of their proximity to circulating blood, do not undergo complete destruction and retain some of their nuclei. It is possible, also, that fibroblastic elements may contribute to myogenesis. Some of the histological appearances in the sections suggest that this may occur, but in no case can it be affirmed with certainty. In this connexion, it may be noted that over 50 years ago Volkmann (1893) concluded that the regeneration of striated muscle always proceeds from the nucleated remnants of old fibres.

As might be expected, the adequacy of intramuscular anastomoses varies in different muscles, and even to some extent in the same muscle in different animals. Thus while in the tibialis anterior and the semimembranosus of the rabbit localized regions of functional devascularization can be produced by partial interruption of main vessels, in the gracilis (where the anastomoses are particularly free) effective devascularization is only produced by ligature of all the main vessels of supply. Again, in the tibialis anterior, while ligature of the lower vessels of supply usually leads to a temporary functional devascularization of the lower part of the muscle, the upper limit of the devascularized region varies from animal to animal, and in exceptional cases complete devascularization of the lower part is not obtained.

It is interesting to note that when all the main vessels of supply to a muscle are interrupted, part of the muscle can still be to some degree vascularized through small vessels which enter it at the site of its attachment. The extent to which this can occur naturally varies with the type of attachment. In the case of the upper attachments of the tibialis anterior and the gracilis, which are broad and fleshy, approximately the upper third of the muscles can gain an effective blood supply from this source, though the lighter intensity of staining with bromo-phenol blue indicates some impairment of the circulation. In the case of the lower attachment of the semimembranosus, which is thin and membranous, effective vascularization only extends into the muscular tissue immediately adjacent to the tendon. On the other hand, it seems that this mode of vascularization is by no means necessary under

* Forbus mentions in his paper that his experiments included the interruption of the blood supply to muscle for a period of 3 hr., but, curiously enough, he does not record the results of these particular experiments.

normal conditions, for we have found that the muscles can be separated from their attachments without any effect on their staining with bromophenol blue. We may note, also, that the interruption of a small main vessel of supply to a muscle may be without apparent effect; for example, ligature of the artery which enters the lower third of semimembranosus does not diminish the intensity with which it is stained by the dye. Lastly, it may be supposed that a muscle which has extensive attachments over most of its length (such as the crureus) would hardly be affected by occlusion of its main vessel of supply, since its circulation could be adequately maintained by small vessels reaching it through its attachments.

The ease with which it is possible experimentally to effect a functional devascularization of part of a muscle by occlusion of one of its vessels of supply, or of a vascular channel in its substance, is evidently of considerable practical significance, for it is clear that injury to a limb may in the same way cause a local devascularization lasting for some days. That this is the case has been demonstrated by our experiments on the effects of gunshot wounds through muscles in the rabbit's leg, and, if the intramuscular anastomoses in human muscles are no more adequate than they are in the rabbit, the same presumably applies to man. A muscle thus affected may be supposed to be particularly susceptible to infection, especially with anaerobic organisms. Thus, in the prophylactic surgical treatment of wounds involving muscles, it is of importance that muscular tissue whose blood supply has been seriously affected should be removed. In order to do this effectively, some knowledge of the vascular patterns within the muscles of the human body is requisite so that the extent of the devascularized tissue can be approximately estimated. Unfortunately, little precise information is yet available on this subject, apart from the source and points of entry of the main muscular arteries, though some data are provided by the radiographs in the papers of Wollenberg (1905) and Campbell & Pennefather (1919). It may be presumed, also, that a knowledge of vascular patterns would be applicable to muscle surgery even in cases where the possibility of sepsis can be eliminated, for, in cases where a muscle has to be cut, it would be advisable, in order to ensure perfect recovery of function, to avoid interruption of the main vascular channels. On the other hand, our experiments on rabbits indicate that under favourable conditions devascularized areas of muscle can subsequently undergo rapid and complete repair by the regeneration of muscle fibres, though it has yet to be shown that this occurs with equal facility in human muscles. It has also to be shown that the newly-formed fibres become finally established as functional elements, and that the regeneration observed is not merely a temporary phenomenon.

SUMMARY

1. Using bromo-phenol blue as an indication of the effective vascularization of muscular tissue, the results of interrupting the blood supply to muscles have been studied experimentally in rabbits.

2. The efficiency of intramuscular anastomoses varies in different muscles. For example, in the case of the tibialis anterior of the rabbit, which receives two main arteries of supply, ligature of the lower artery leads to a temporary devascularization of the distal half of the muscle with necrosis of muscle fibres. Partial interruption of intramuscular vascular channels in the tibialis anterior or the semimembranosus leads to localized areas of devascularization.

3. Necrotic areas in the devascularized muscles of rabbits are rapidly replaced by regenerated muscle fibres, leading to a partial reconstitution of the muscle.

4. Experimental gun-shot wounds involving the thigh muscles of rabbits may give rise to circumscribed patches of devascularization extending some distance from the track of the missile.

5. The practical importance of a knowledge of intramuscular vascular patterns in human muscles is emphasized.

We wish to record our thanks to Mr E. Thompson for his expert assistance in the preparation of histological material, and to Mr W. Chesterman for the microphotographs which illustrate this paper.

REFERENCES

Brooks, B. (1922). *Arch. Surg., Chicago*, **5**, 188.
Brooks, B. (1924). *J. Bone Jt Surg.* **6**, 326.
Brooks, B. & Martin, K. A. (1923). *J. Amer. Med. Ass.* **80**, 1678.
Brooks, B., Johnson, J. A. & Kirtley, Jr. (1934). *Surg. Gynec. Obstet.* **59**, 496.
Campbell, J. & Pennefather, C. M. (1919). *Lancet*, **1**, 294.
Durante, G. (1902). *Manuel d'histologie pathologique*. Paris: Cornil and Ranvier.
Forbus, W. D. (1926). *Arch. Path.* **2**, 318, 486.
Garbé, K. (1906). *Verh. Ges. Dtsch. Naturf. Arzt.* **78**, 202.
Gilding, H. P. (1932). *J. Physiol.* **74**, 34.
Griffiths, D. L. (1940). *Brit. J. Surg.* **28**, 239.
Heidelberg, v. M. (1878). *Arch. Exp. Path. Pharmak.* **8**, 335.
Holman, E. & Edwards, M. E. (1927). *J. Amer. Med. Ass.* **88**, 909.
Jepson, P. N. (1926). *Ann. Surg.* **84**, 785.
Middleton, D. S. (1930). *Brit. J. Surg.* **18**, 188.
Meyenburg, H. v. (1929). *Handb. Spez. path. Anat. Hist.* Berlin: F. Henke and O. Lubosch, J. Springer.
Rous, P. & Drury, D. R. (1929). *J. Exp. Med.* **49**, 435.

ROUS, P. & GILDING, H. P. (1929). *J. Exp. Med.* **50**, 189.
ROUS, P., GILDING, H. P. & SMITH, F. (1930). *J. Exp. Med.* **51**, 807.
SPALTEHOLZ, W. (1888). *Abh. sächs. Ges. (Akad.) Wiss.* **24**, 509.
TRUETA, J. (1943). *Principles and Practice of War Surgery*. London: Hamish Hamilton.
VOLKMANN, R. (1893). *Beitr. path. Anat.* **12**, 233.
WILSON, W. C. (1932). *Brit. J. Surg.* **20**, 393.
WOLLENBERG, A. (1905). *Z. orthop. Chir.* **14**, 312.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Sagittal section through approximately the middle two quarters of tibialis anterior 4 days after ligature of both main arteries of supply. The sharp line of demarcation between the normal muscular tissue in the upper third, which still remains vascularized, and the devascularized tissue, is shown at *a*. Below this level, the muscle is almost entirely in process of degeneration, except for occasional surviving fibres alongside blood vessels and at the surface in contact with the epimysium. ×6.

Fig. 2. Sagittal section through approximately the middle two-quarters of tibialis anterior 11 days after ligature of both main arteries of supply. The junctions of persisting old muscular tissue and regenerated muscular tissue in the upper and lower thirds of the muscle are indicated approximately by the arrows marked *a*. Between these junctional zones the muscle is composed entirely of degenerated and regenerating tissue, except for isolated and scattered old fibres which have survived alongside main vessels in the muscle and on the surface in contact with the epimysium. A relatively large circumscribed area of degenerated muscular tissue is seen on the posterior surface (*b*), and smaller areas are seen in the middle of the muscle to the right of this. These areas are in process of rapid removal by granulation tissue into which myoblastic elements are streaming from above and below. Near the anterior surface of the muscle (to the right in the photograph), regenerated fibres have grown through the whole extent of the devascularized part of the muscle. ×6·5.

Fig. 3. Sagittal section through approximately the middle two quarters of tibialis anterior 14 days after ligature of both main arteries of supply. The devascularized part of the muscle is now almost completely reconstituted by the growth of regenerated muscle fibres and it is not possible to determine precisely the boundaries between the original muscular tissue and newly formed tissue. In the central axis of the muscle, numerous old fibres have survived. On either side of these, muscular tissue of finer texture is seen in which the fibres run an undulating course. These fibres have all been formed by regeneration, and no degenerated fibres remain. ×10.

PLATE 2

Fig. 4. Longitudinal section through the lower half of the tibialis anterior 2 days after ligature of both the main arteries of supply. The fibres in this devascularized part of the muscle preserve their cross-striations, but they are devoid of nuclei. There is an interfibrillar cellular exudate and endomysial oedema. ×350.

Fig. 5. Longitudinal section of the lower third of semimembranosus 5 days after devascularization. Above are seen degenerated old fibres undergoing a hyaline necrosis. From their stumps myoblastic elements appear to be streaming out into the fibroblastic tissue below. ×126.

Fig. 6. Myoblasts in the fibroblastic tissue which has replaced the necrotic muscular fibres in the devascularized lower portion of tibialis anterior after ligature of both main arteries of supply. ×350.

Fig. 7. A further stage in the development of new muscle fibres. The myoblasts have become elongated to form 'myotubes' and each contains a row of closely packed nuclei. To the right are seen some surviving old muscle fibres. ×350.

Fig. 8. This microphotograph illustrates a later developmental stage in the formation of new muscle fibres in the devascularized tibialis anterior. The myoblasts have undergone considerable elongation and their nuclei have become spaced out down the centre in clumps of three or four. Longitudinal fibrillation is quite distinct at the periphery of the fibres.

Fig. 9. At this stage of development, from a tibialis anterior 8 days after devascularization, the newly formed muscle fibres have increased in thickness and show a faint cross-striation. Some of the nuclei are still arranged in clumps, but many have become displaced to a peripheral position. ×350.

PLATE 3

Fig. 10. Longitudinal section of newly formed muscular tissue from the lower part of a tibialis anterior 14 days after ligature of both main arteries of supply. The fibres show longitudinal fibrillae and a definite cross-striation. The nuclei are disposed singly, and most of them are situated near the surface of the fibres. ×350.

Fig. 11. Transverse section of the junction of the middle and lower thirds of the tibialis anterior 3 weeks after ligature of both main arteries of supply. Note the circular contour of the fibres, the presence of central nuclei, and the spaces which separate the fibres from their endomysial sheaths. ×350.

Fig. 12. Longitudinal section of the central region of the lower part of tibialis anterior 5 days after ligature of both main arteries of supply. Alongside the central vessels (to the left) are seen some persisting old fibres of normal appearance. To the right are swollen degenerated fibres undergoing fragmentation. ×103.

Fig. 13. Longitudinal section from the surface of the middle third of tibialis anterior 14 days after ligature of both main arteries of supply. Newly regenerated fibres are shown, and above is one surviving old fibre lying in contact with the epimysium. ×350.

Fig. 14. Longitudinal section of semimembranosus 5 days after devascularization. Above are seen segments of degenerated muscle fibres invested with granulation tissue, and below are numerous fusiform myoblasts which appear to be derived from the remains of the degenerated fibres. ×126.

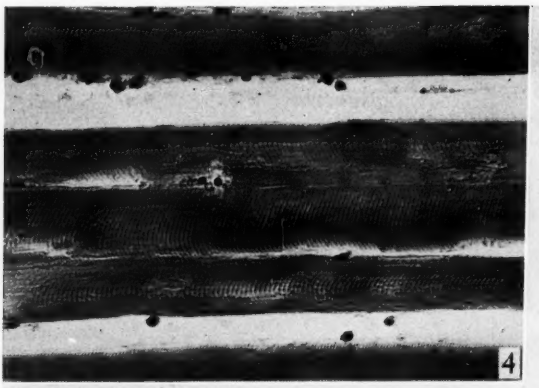
Plate 4

Fig. 15. Medial aspect of the thigh of a rabbit which had been injected with bromo-phenol blue and killed 60 hr. after a gun-shot wound through the adductor magnus. The track of the shot is seen in the middle of the muscle and below this is a well-defined rectangular area which in the photograph appears much darker than the surrounding muscle. This area was quite unstained by the bromo-phenol blue and was found to be effectively devascularized. ×1.

Fig. 16. Section through the adductor magnus shown in Fig. 15, at the junction of the devascularized and intact parts of the muscle. Above is the normal muscular tissue and below the devascularized area. In the latter the muscle fibres are swollen, stain faintly, and have lost their nuclei. At the junction of the normal and devascularized areas, and also on the surface of the muscle, are zones of active necrosis in which the disintegrating fibres stain deeply with haematoxylin. ×16.

Fig. 17. Section showing the junctional zone of normal and devascularized muscle, taken in this case from the tibialis anterior 2 days after ligature of both main arteries of supply. The muscle fibres in this zone are necrotic and undergoing rapid disintegration. ×350.

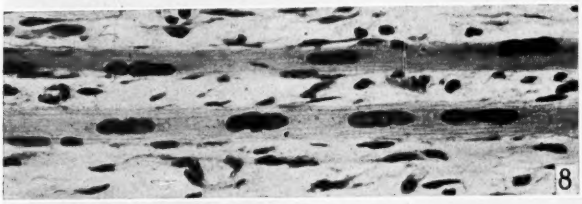
a
1
a
a
b
a
a
2
3


4

5

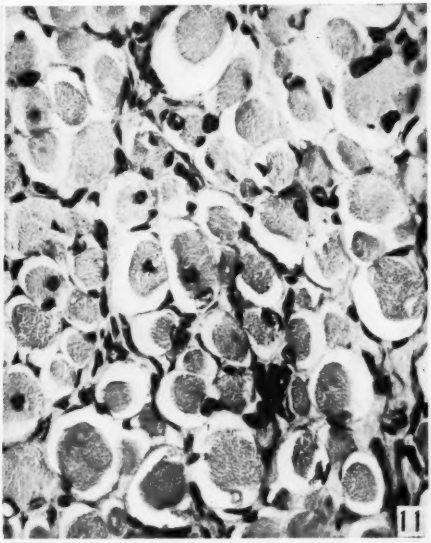
6

7

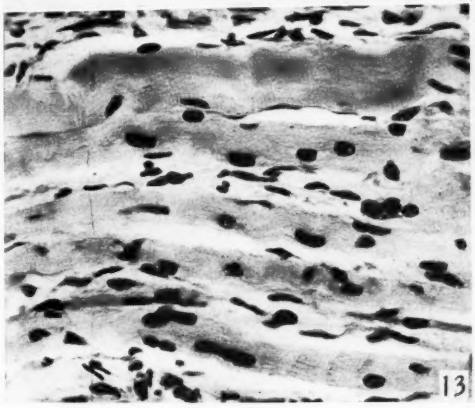

8

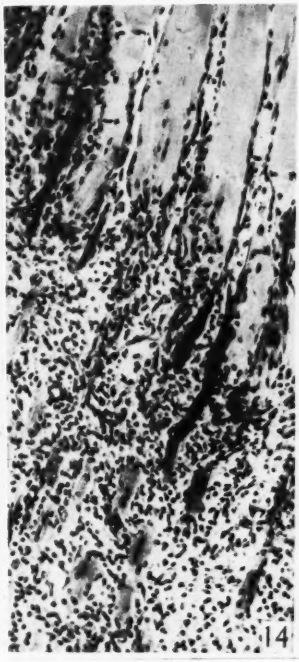
9

10

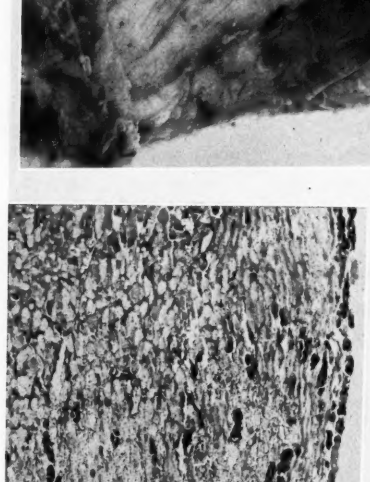

11

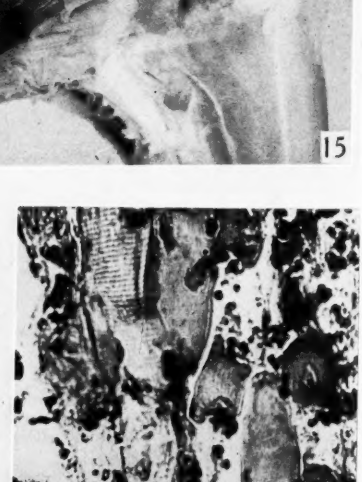
12


13


14

15


16


17

THE MENINGEAL RELATIONS OF THE HUMAN HYPOPHYSIS CEREBRI

By S. SUNDERLAND, *Department of Anatomy and Histology, University of Melbourne*

Opinions vary widely concerning the meningeal relations of the hypophysis cerebri. In general the fundamental point at issue is whether or not the arachnoid, pia mater and subarachnoid space continue through the aperture in the diaphragma sellae to surround the gland in the sella turcica and if so the extent to which this occurs. This is a subject of some importance, since the paths of secretion of the gland are ultimately determined by its structure and relations. Thus direct and generalized contact with the subarachnoid space would make it possible for the secretions to be poured into the space and from there to be diffused over the entire cerebrospinal fluid pathway, whereas if that part of the gland below the diaphragma sellae, and this represents the major portion of the gland, is completely and securely sealed off from the space then no such pathway can exist.

The most striking feature of the published reports on this subject is the predominance of studies on the meningeal relations of the gland in lower mammals and the few occasions on which the human hypophysis has been the subject of study. Investigations on human material appear to have been confined to the recent studies of Roussy & Mosinger (1934) and of Wislocki (1937) on embryological material, while references to the arrangement in the adult are limited to the brief accounts contained in the works of Key & Retzius (1876), Erdheim (1904) and Davidoff & Dyke (1937), who were not specifically concerned with this particular problem. As a consequence text-book descriptions and illustrations of the disposition of the leptomeninges in man, and the physiological and pathological implications based thereon, have often been unduly influenced by observations recorded on lower mammals. Though animal studies and experimentation admittedly confer certain advantages on an investigation it would be unfortunate if, in the study of human problems, we were content to rely solely on assumptions and speculations based on an examination of subhuman material without endeavouring to establish or confirm the details of the arrangement in man. The investigation which forms the subject of this report was concerned solely with the meningeal relations of the human pituitary gland and was undertaken with a view to determining, in particular, the relations of the gland to the aperture in the diaphragma sellae and to the subarachnoid space.

LITERATURE

The following reference from Key & Retzius (1876), whose book is not available in this country, has been taken from Hughson's (1924) paper. Key & Retzius described the spread of the injection mass in the subarachnoid space as follows: 'Tafel vii, Fig. 1: Around the peduncle of the hypophysis can be seen the injection mass which has gone through the dura and spreads out between the hypophysis and arachnoid which spans the sella turcica. Fig. 2: In the cross-section of the hypophysis one sees on its upper surface, between it and the dura which bridges it, that the injection mass has penetrated the space as in the preceding figure.' Their demonstration of a space between the gland and the diaphragma sellae is of especial significance. It is a feature which appears to have been missed by subsequent investigators with the exception of Davidoff & Dyke (1937), who claim to have observed a similar space beneath the diaphragm in their encephalographic studies. Further reference to this point will be made later. According to Erdheim (1904) the capsule of the gland is intimately related to the dural lining of the sella turcica. He illustrates and describes the arachnoid bending down in front of the infundibulum and then turning back, without reaching the diaphragma sellae, to become inserted on the middle of the anterior surface of the infundibulum. Lewis & Knavel (1913) claim that occasionally 'the arachnoid passes below the diaphragm of the sella to spread over the surface of the hypophysis'. Stendell (1914), Woerdeman (1918) and Cowdry (1922) describe or illustrate an extension of the pia mater, but no other membrane, into the fossa to surround the neural lobe only. Hughson (1924) claims to have demonstrated, in the dog and cat, by methods involving the introduction of Prussian blue reagents into the subarachnoid space and their subsequent precipitation in that situation, that this space completely invests the gland. His method, however, involved manipulating the cerebrospinal fluid pressures over abnormal limits in order to promote the widest possible dispersal of the injection fluid. In one set of experiments this was drawn into the subarachnoid space following reduction of the cerebrospinal fluid pressure by the intravenous injection of 30% sodium chloride, while in another it was introduced under pressure. According to Bailey

(1932) 'the dura mater also lines the sella so that it comes thus to form a continuous capsule within which the subarachnoid space extends everywhere except at the extreme posterior extremity where the blood vessels enter the posterior lobe'. He has apparently relied on Hughson's description in the dog for his information, though the accompanying illustration is one modified from Atwell (1926) who, however, made no attempt to represent the pia or arachnoid inside the sella. Brander (1932) could find no trace of a subarachnoid space around the posterior lobe or around the pituitary as a whole. He believed that there was sufficient evidence 'to justify a definite statement that at no stage of post-uterine life is such a space other than hypothetical'. Roussy & Mosinger (1934) examined the meningeal relations of the gland in human, dog and cat embryos. Their account of the disposition of the dura corresponds with that given by other investigators. They describe the pia as dividing, on reaching the anterior surface of the infundibulum at the base of the brain, into two sheets which separate to enclose the pars tuberalis. The posterior sheet descends between the infundibulum and the pars tuberalis to enter the gland where it loses its identity in the supporting tissue. The anterior sheet descends in front of the pars tuberalis to reach and fuse anteriorly with the sharp dural margin of the aperture in the diaphragm. Thus above the diaphragm the pars tuberalis is regarded as being an intrapial structure. The pia descends on the posterior surface of the infundibulum to become continuous with the posterior margin of the aperture at the level of the diaphragm. They deny the existence of a perihypophysial subarachnoid space and claim that the arachnoid inserts on to that layer of tissue which constitutes the upper part of the capsule of the gland. It is difficult, however, to reconcile their account with the following statement contained in their report: 'La couche pie-mérienne de la méninge molle prend part, dans une forte proportion, à la constitution de l'espace péri-hypophysaire. Ces faits expliquent que sous l'influence de pressions intra-arachnoidiennes élevées, le liquide cephalorachidien puisse pénétrer dans cet espace.' Schwartz (1936) repeated Hughson's experiments on the dog but corrected the abnormal pressures obtaining in the subarachnoid space in the latter's experiments by replacing small quantities of cerebrospinal fluid with a dilute saline solution of India ink. Under these conditions, which maintained the cerebrospinal fluid pressure at a constant level, he was unable to demonstrate any extension of the subarachnoid space around the gland, the dura being everywhere adherent to the hypophysial capsule. Wislocki (1937), from observations made on serial sections through the hypophysial region in adult rats, rabbits, cats and rhesus monkeys, concluded that 'the capsule of the pituitary gland, the intrasellar dura and the adjacent periosteum constitute a continuous fused lamina, precluding extension into the sella of either the subarachnoid or subdural space'. He later (1937) extended his studies to include the human hypophysis which he examined in foetal material. His conclusions were substantially the same, namely, that 'the dura developing around the body of the hypophysis and forming the sellar diaphragm fuses with the surface of the anterior and neural lobes and hence prevents permanently the development of pia arachnoid or of a subdural space around the body of the pituitary'. Davidoff & Dyke (1937), in their account of the subarachnoid cisterns as outlined by air in encephalograms, describe an extension of the cisterna chiasmatis ventrally to become continuous with an hypophysial subarachnoid space. According to them this space 'is formed by a sheath of arachnoid tissue, which enters with the infundibulum of the hypophysis and spreads out ventral to the dural diaphragm of the sella turcica to enclose the pituitary gland. This forms a narrow subarachnoid space except directly beneath the diaphragma sellae where it attains a width of about 1 mm. The arachnoid around the hypophysis is closely adherent to the dura so that in this region there is no subdural space.'

A very important and significant feature which appears to have escaped attention is the size of the aperture in the diaphragm relative to the dimensions of the infundibulum and pars tuberalis which it transmits, for this feature must determine the extent to which the upper surface, at least, of the gland comes into relationship with the leptomeninges and subarachnoid space. The impression gained from a study of the descriptions and illustrations favouring the total exclusion of the leptomeninges from the fossa is that the aperture is barely large enough to transmit the infundibulum and pars tuberalis, which completely fill it, so that the fossa and its contents are sealed off superiorly by the thick dural diaphragm. On the basis of such an arrangement Rioch (1938) believes that the pars tuberalis alone is favourably situated to discharge its secretions into the subarachnoid space.

MATERIAL AND METHODS

The observations recorded in this paper are based on the macroscopic examination and dissection under a microscope of the pituitary fossa and its contents in seventy-six adult dissecting room subjects together with the histological examination of this region in seven children aged 1, 2½, 3, 4½, 9 and 12 months and 9 years respectively. In the post-mortem material the entire bony fossa together with the gland and the interpeduncular region of the base of the brain was carefully removed *en bloc*

without disturbing the normal anatomical relations. The mass was then fixed, decalcified, embedded in celloidin and sectioned serially in the sagittal plane at 50 μ. The sections were stained with haematoxylin and eosin and by van Gieson's method. No attempt was made to investigate cytological detail.

OBSERVATIONS

Arrangement in the infant

The anatomical arrangement in infants and young children will be described first. The relations of the meninges to the gland in these specimens are illustrated in Pl. 1, fig. 1.

Dural relations. The sella turcica is shallower in infants than in adults. It is lined throughout by the dura which spans the fossa superiorly, between the clinoid processes, to form a diaphragm which carries a central, round or oval aperture; where the dura comes into contact with the gland it assists in the formation of its capsule. This aperture is usually considerably larger than the infundibulum and the pars tuberalis which it transmits. Only occasionally is the dura firmly applied to the structures passing through it. As a result, a variable but usually extensive portion of the upper surface of the gland is left uncovered by the dura and the gland frequently bulges upwards through the deficiency owing to the shallow nature of the fossa. Usually the posterior rim of the aperture is closely applied to the stalk and the anterior margin is removed from it by some distance. Consequently it is the superior surface of the pars anterior and pars tuberalis which is so exposed.

Pial relations. The arrangement observed corresponds with that described by Roussy & Mosinger. The pia descends along the stalk, after enclosing the pars tuberalis, to reach the upper surface of the gland. Here the anterior layer is reflected forwards, blending with the perihypophysial capsule, until it meets the margin of the dural aperture with which it becomes continuous. It is not possible to identify precisely where the pia terminates and the dura begins, and some might even argue that it is the dura which thins out posteriorly to blend with the pia at the site of attachment of the stalk to the body of the gland. The histological sections and the arrangement present in the adult do not favour such an interpretation. Regardless, however, of the manner in which the meningeal layers are identified, the significant feature is that there is a large portion of the upper surface of the gland in front of the stalk which is very thinly covered. Posteriorly the pia descending along the stalk blends in the same imperceptible manner with the posterior dural margin of the aperture which is itself usually closely applied to the stalk. In this manner the pial reflexions from the stalk seal that portion of the aperture in the diaphragma sellae which is not occupied by the infundibulum and pars tuberalis. Though occasionally the pia could be traced along the under-surface of the diaphragm for a short distance before blending with the perihypophysial capsule, this layer usually loses its identity immediately it reaches the margin of the aperture. In no instance could a discrete layer of pia mater be traced around the gland.

Arachnoidal relations. The arrangement observed corresponds with that described by Erdheim. The arachnoid, with a well-developed zone of subarachnoid tissue, envelops the chiasma and descends anterior to the stalk to reach the pial reflexion covering the upper surface of the gland. It then passes back to surround the stalk immediately above its attachment to the gland and continues on to outline the floor of the interpeduncular cistern. Thus the arachnoid hangs as an apron-like fold anterior to the infundibulum, and the posterior leaf is arranged as a collar about it. Though the apex of the fold usually reaches or approximates to the pia covering the upper surface of the gland, it does not usually extend below the level of the free margin of the dural aperture because this, in addition to being covered by the pia mater, is usually filled by the gland. In the histological sections shrinkage has carried the arachnoid away from the pia covering the deficiency in the diaphragm, but its intimate relationship to the pia in this situation can be readily confirmed by dissection.

Conclusions. The only tissue covering the gland in the sella turcica is the dura which blends with the perihypophysial capsule. The aperture in the diaphragm is usually much larger than the structures it transmits, and the residual deficiency thereby created is filled in by the pia which is reflected from the stalk to the margins of the aperture. The consequence of the dural arrangement is to leave, in the majority of cases, a variable but usually extensive portion of the upper surface of the gland, generally that anterior to the stalk, covered only by pia mater. Immediately above this is the apron-like fold of arachnoid which hangs from the chiasma in front of the stalk to reach the pia covering the upper surface of the gland. Thus in infancy and early childhood the disposition of the meninges is such that the subarachnoid space is related to that portion of the gland which is applied to the dural aperture and which usually involves both the pars tuberalis and pars anterior.

Arrangement in the adult

In the adult (Pl. 1, figs. 2–6) the arrangement just described may persist though it is often modified in some measure by disproportionate growth between the sella and the gland. With the relative descent of the gland in the fossa, the pia mater, which is firmly attached to its upper surface where it fills the aperture, is pulled through the opening so that

the dural margins of the latter become more sharply defined. This process may occur to such a degree that the sharp margin of the aperture comes to surround a central pial depression into which the apron-like fold of the arachnoid may project. The extreme form of this condition is seen in old age when atrophy has still further reduced the size of the gland. In such cases the pial depression is converted into a very deep fossa which extends well into the sella and into which the arachnoid and subarachnoid space are herniated. In this way a meshwork of arachnoidal tissue bathed in cerebrospinal fluid spreads over the upper surface of the gland. If the factors responsible for the altered relationships in the fossa continue to operate, it is possible for the gland in the fossa to become almost, if not completely, surrounded by the leptomeninges though such an advanced condition was not seen in this series. Only, however, in these circumstances do the arachnoid and subarachnoid space extend into the sella turcica. Such an encroachment is facilitated, extended and accelerated by further atrophy of the gland or by a continued disproportionate increase in size of the fossa. This is apparently the condition represented in the illustrations of Key & Retzius in which their injection mass in the subarachnoid space had penetrated into a space between the gland and the dura which bridges it. It also accounts for the hypophysial subarachnoid space which Davidoff & Dyke claim to have demonstrated by encephalography.

The dura of the diaphragm was tightly applied to the infundibulum and pars tuberalis in only 20 % of the specimens examined.

CONCLUSIONS

At all ages a variable extent of the superior surface of the gland, more commonly that portion anterior to the stalk, is related directly to the pia, arachnoid and subarachnoid space. In the foetus, and in infancy and childhood, this area is limited to that portion of the gland which is directly applied to the aperture in the diaphragma sellae. It is consequently determined by the size of the aperture but is usually sufficiently extensive to involve both pars tuberalis and pars anterior. This area may be extended as the gland and the firmly attached pia alter their relationship to the fossa and drop away from the diaphragma sellae. This has the effect of permitting the arachnoid and subarachnoid space to descend through the aperture, and these may ultimately extend over the entire upper surface of the gland or even completely around the body should the factors responsible for the altered relationships in the fossa continue to operate.

The type and amount of glandular tissue establishing a relationship with the subarachnoid space, in the absence of any intervening dura, varies over very wide limits but depends on the size of the aperture in the diaphragma sellae and the degree of extension of the leptomeninges into the fossa. This extension may be sufficiently extensive to involve all parts of the body of the gland though the pars tuberalis and pars anterior are most commonly affected. Under these conditions it becomes possible for pituitary tissue in the fossa, other than that of the pars tuberalis, to secrete directly into the subarachnoid space. In view, however, of the thickness of the perihypophysial capsule surrounding the body of the gland it is extremely unlikely that such a pathway exists. On the other hand, the neural and tuberal elements of the stalk of the gland lack this thick capsule above the level of the diaphragm. In this situation they are ensheathed only by a very thin coating of pia so that the diffusion of their products into the subarachnoid space represents, on anatomical grounds at least, a possible pathway of secretion.

SUMMARY

1. The relationship of the meninges to the human hypophysis has been examined macroscopically in a series of seventy-six adult dissecting room specimens and microscopically in seven post-mortem specimens, obtained from infants, in which the gland and the surrounding structures, bony and neural, had been removed *en bloc* and prepared for histological examination.

2. Attention is drawn to the large size of the aperture in the diaphragma sellae which is usually only partly occupied by the infundibulum and pars tuberalis. The residual lumen is closed and the fossa completely sealed off by the reflexion of the pia from these structures to the margins of the aperture. Only occasionally is the dura firmly applied to the structures passing through it. This pial reflection assists in the formation of the perihypophysial capsule.

3. In infancy and childhood there is normally no arachnoid membrane below the level of the dural aperture, owing to its closure by the pia and the gland, and consequently there is no extension of the subarachnoid space into the pituitary fossa.

4. In adult life, and more particularly when there is atrophy of the gland with advancing age, the gland sinks into the fossa. This has the effect of enabling the pia, arachnoid and subarachnoid space to descend through the aperture and ultimately to extend over the entire upper surface of the gland or even completely around the body should the factors responsible for the altered relationships in the fossa continue to operate.

5. The type and amount of glandular tissue establishing a relationship with the subarachnoid space, in the absence of any intervening dura, vary over

Fig. 1

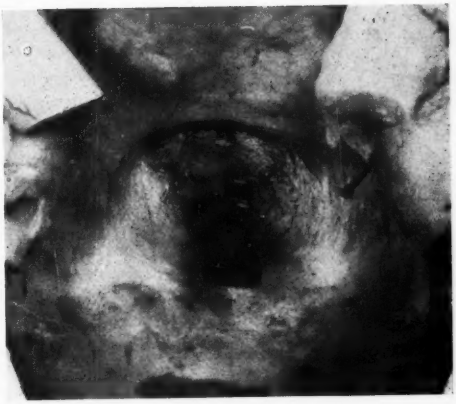


Fig. 2

Fig. 3

Fig. 4

Fig. 5

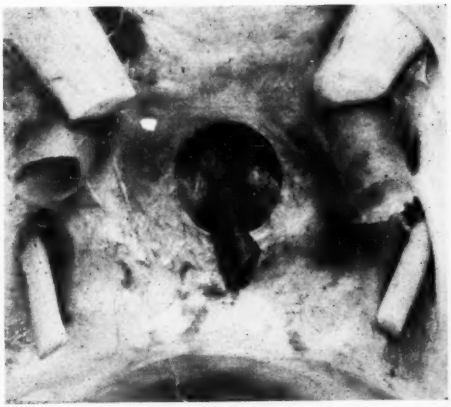


Fig. 6

UNIV. OF MICH.

very
aper
of pe
The
path

ATWE
BAILE
2nd
BRAN
COWD
705
DAVII
End
ERDH
Hir
HERR
HUGH
232
KEY,

Fig. 1.
its
the
Fig. 2
fron
the
app
defi
Fig. 3
illus
rou
Fig. 4
afte

very wide limits but depend on the size of the aperture in the diaphragma sellae and the degree of penetration of the leptomeninges into the fossa. The bearing of the anatomical arrangement on the paths by which the secretory products of the different parts of the gland may be discharged is briefly discussed.

The author's thanks are due to Mr L. Preston for the photographs which accompany this paper.

REFERENCES

ATWELL, WAYNE, J. (1926). *Amer. J. Anat.* **37**, 159.

BAILEY, PERCIVAL (1932). Cowdry's *Special Cytology*, 2nd ed., **2**, 773. New York.

BRANDER, JOHN (1932). *J. Anat.* **66**, 202.

COWDRY, E. V. (1922). *Endocrinology and Metabolism*, **1**, 705. New York.

DAVIDOFF, L. M. & DYKE, C. G. (1937). *The Normal Encephalogram*. Philadelphia.

ERDHEIM, J. (1904). *Über Hypophysenganggeschwulste und Hirncholesteatome*. Wien.

HERRING, P. T. (1908). *Quart. J. Exp. Physiol.* **1**, 121.

HUGHSON, WALTER (1924). *Bull. Johns Hopk. Hosp.* **35**, 232.

KEY, A. & RETZIUS, G. (1876). *Anatomie des Nerven-systems und des Bindegewebe*. (Cited by Hughson.) Stockholm.

LEWIS, DEAN & KNAVEL, A. B. (1913). Keen's *Surgery*, vol. **6**. Philadelphia.

RIOCH, D. McK. (1938). Paths of Secretion from the Hypophysis. In *The Pituitary Gland*. Baltimore.

ROUSSY, G. & MOSINGER, M. (1934). *Rev. Neurol.* **1**, 568.

SCHWARTZ, HENRY GERARD (1936). *Anat. Rec.* **67**, 35.

STENDELL, WALTER (1914). Bd. **8** in Oppel's *Lehrb. d. vergleich. mikr. Anat. d. Wirb.* Jena: Fischer.

WISLOCKI, GEORGE BERNAYS (1937). *Anat. Rec.* **67**, 273.

WISLOCKI, GEORGE B. (1937). *Amer. J. Anat.* **61**, 95.

WOERDEMAN, MARTIN W. (1918). *Ned. Tijdschr. Geneesk.* **1**, 215.

EXPLANATION OF PLATE

Fig. 1. Mid-sagittal section through the pituitary fossa and its contents in a 12 months old infant to demonstrate the meningeal relations of the gland and its stalk. ×7.

Fig. 2. Photograph of the pituitary region in the adult from above. In this specimen the dura almost reaches the infundibulum and the body of the gland is closely applied to the diaphragm so that there is no clearly defined margin to the aperture and no pial depression.

Fig. 3. Photograph of the pituitary region from above illustrating the meshwork of arachnoid tissue which surrounds the infundibulum and covers the diaphragma sellae.

Fig. 4. Photograph of the same specimen shown in fig. 3 after the arachnoid has been carefully dissected away. The aperture in the diaphragm is visible together with the saucer-shaped depression caused by the displacement of the pia mater into the fossa.

Fig. 5. This specimen represents a further stage in the process of shrinkage of the gland into the fossa. The margin of the aperture is more clearly defined and the pial depression deeper.

Fig. 6. This photograph illustrates the arrangement present when an atrophic pituitary gland is only partly occupying a fossa of normal dimensions. Both pia and arachnoid extend into the fossa and around the gland to outline a large and extensive subdiaphragmatic perihypophysial subarachnoid cistern.

POST-NATAL GROWTH-CHANGES IN THE CARDIAC VENTRICLES OF THE ALBINO RAT

By J. A. KEEN, *Department of Anatomy, University of Cape Town*

In all young mammals differential growth changes in the cardiac ventricles begin immediately after birth, to adjust the heart to the changed condition of the circulation. To these changes, as demonstrated by a small series of human infant hearts, attention was called previously (Keen, 1942). This series was limited to single specimens only for the various constituent age groups, hence individual variation could not be excluded; moreover, such observations upon autopsy material may be unrepresentative of normal cardiac anatomy by reason of circulatory derangements associated with the terminal disease. Hence a systematic investigation seemed desirable of ventricular growth changes in some laboratory mammal, and for this purpose the albino rat was chosen.

MATERIAL AND METHODS

The hearts were used of albino rats varying in age from birth to 14 days: the number of hearts for each age group is indicated in Table 1. Several litters being simultaneously available for each age group, the components of such were made as representative as possible by the inclusion of not more than two specimens from any one litter. The animals were killed with chloroform or coal-gas, without any resultant appreciable difference. After fixation, hand-cut cross-sections of the ventricular portions of the hearts were made, midway between cardiac apex and atrio-ventricular sulcus; the sections were laid on glass slides and drawn by camera lucida at a magnification of $\times 15$. A certain difficulty was experienced during such drawing (particularly with hearts fixed in systole) in distinguishing between the ventricular musculature proper and the intraventricular papillary projections. Fortunately most of the hearts were fixed in diastole, with clearly defined ventricular cavities. No demarcation was observable in the interventricular septum between the conjoined walls of right and left ventricles, so the thickness of the outer ventricular walls was utilized as a guide to arbitrary division of the septal area into right and left ventricular components. At each end of this arbitrary dividing line certain small areas defied allocation and were therefore omitted from the calculations (see Fig. 1). The cross-sectional areas of the ventricle walls were measured with a planimeter, and their ratios were determined: minor inaccuracies in the ratio may have arisen from the heart sections not having been made in invariably corresponding planes or levels and from the distinction between ventricle wall and papillary muscle being often approximate only. The average body weight of the albino rat during the first 14 days of post-natal life (see Table 1) was determined by weighing four rats from one litter every 24 hr. Donaldson (1924) gives the average weight of the newborn rat as 4·6 g. and that of the 13-day-old rat as 17·2 g., but neither he nor other writers record the average weight of animals of intermediate age. Measurements are provided (Table 1) for the hearts of 120 young rats: the numbers comprising the various age groups are shown, together with the body weights. The measurements of individual hearts are presented in Table 2.

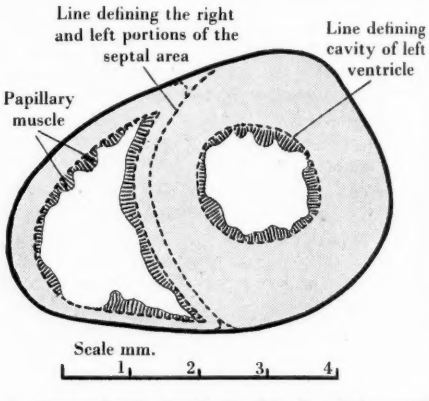


Fig. 1. Camera-lucida sketch of a thin slice of the ventricle of the heart, enlarged about 15 ×: rat aged 12 days.

Planimetric ratios between right and left ventricular walls. The hearts of the first age group studied came from rats killed within 2 hr. after birth. In ten such hearts the L : R ventricular wall ratio varied between 0·9 and 1·2, with a mean of 1·1. On the average there was already present a very slight preponderance of the left ventricular muscle. Later, the ratios increased, the left ventricular wall thickening rapidly relative to the right. A few 3-day, and many of the 4-day, specimens showed planimetric L : R ratios of 2·1 or more, the mean for the

Table 1. *Showing the body weight of rats, the average cross-sectional areas of the two ventricles and the L : R ventricle ratios: from birth to* 14 *days and in the full-grown rat*

Age	Number of rats weighed (one litter)	Average body weight g.	Number of hearts measured	Average cross-sectional areas of the ventricular walls: Left ventricle sq.mm.	Right ventricle sq.mm.	Mean L : R ratio
At birth	—	—	10	2·3	2·1	1·1
1 day	4	5·5	20	2·3	1·8	1·3
2 days	4	6·0				
3 days	4	7·0	20	4·2	2·2	1·9
4 days	4	8·0				
5 days	4	9·4	20	5·7	2·5	2·3
6 days	4	10·4				
7 days	4	12·0	10	5·3	2·1	2·5
8 days	4	13·2				
9 days	4	14·5	10	6·3	2·3	2·7
10 days	4	16·5				
11 days	4	17·7	10	7·9	2·7	2·9
12 days	4	19·2				
13 days	4	20·7	10	9·1	3·0	3·0
14 days	4	22·5				
Full grown (150 days +)		200 +	10	32·9	9·6	3·4

Table 2

A. *Cross-sectional areas of the muscle wall of the left and right ventricles of the rat's heart at various ages after birth, in sq. mm.*

Age																					Mean of areas	Mean ratio
At birth:																						
L.V.	1·9	1·4	1·4	2·3	2·3	1·6	3·1	2·8	2·6	3·8											2·3	1·1
R.V.	2·1	1·4	1·4	1·9	2·1	1·6	2·8	2·4	2·6	3·2											2·1	
1–2 days:																						
L.V.	2·2	3·1	2·4	2·2	1·9	2·0	1·3	2·3	2·3	2·3	3·3	2·5	1·9	3·0	2·7	2·5	2·3	2·3	2·5	2·2	2·3	1·3
R.V.	2·0	2·3	1·6	2·0	1·5	1·6	1·5	1·7	1·3	1·9	1·9	2·4	1·1	2·3	1·6	2·1	1·8	1·6	1·8	1·8	1·8	
3–4 days:																						
L.V.	4·3	5·2	3·0	2·7	2·9	4·1	5·0	5·4	5·1	5·5	4·6	4·1	2·9	3·9	4·3	4·6	4·7	3·8	4·3	5·5	4·2	1·9
R.V.	2·2	2·7	1·5	2·1	1·4	2·3	2·9	2·6	3·0	3·5	2·3	2·5	1·8	1·7	1·8	2·1	2·1	1·7	2·9	2·3	2·2	
5–6 days:																						
L.V.	4·5	4·7	4·0	4·9	6·1	5·3	4·2	4·4	5·2	6·0	5·3	6·9	8·0	5·0	5·3	5·0	5·9	5·3	8·7	10·0	5·7	2·3
R.V.	1·8	1·8	2·2	2·2	3·2	2·6	1·7	2·2	2·5	2·6	2·1	2·3	3·1	1·9	2·1	2·5	2·9	2·7	3·2	4·4	2·5	
7–8 days:																						
L.V.	4·4	6·5	6·2	6·2	5·1	5·7	3·4	5·5	4·7	5·0											5·3	2·5
R.V.	2·1	2·3	2·5	2·7	2·1	2·3	1·4	2·2	1·9	1·6											2·1	
9–10 days:																						
L.V.	4·4	6·8	5·3	8·4	4·9	7·8	4·0	6·0	6·8	8·3											6·3	2·7
R.V.	1·8	3·2	2·1	2·9	1·4	2·9	1·4	2·3	2·5	3·2											2·3	
11–12 days:																						
L.V.	5·5	6·4	8·9	8·0	8·6	7·6	8·3	10·1	9·2	6·5											7·9	2·9
R.V.	2·3	2·2	2·7	3·2	2·7	2·5	2·6	3·6	3·4	2·3											2·7	
13–14 days:																						
L.V.	9·2	7·8	10·1	6·7	8·9	10·2	9·9	9·5	9·9	9·3											9·1	3·0
R.V.	2·8	2·5	3·9	2·3	2·7	3·2	3·0	3·4	3·3	3·3											3·0	
Full grown (150 days +):																						
L.V.	27·2	34·0	30·2	36·1	40·7	42·5	32·5	29·7	32·2	24·4											32·9	3·4
R.V.	8·0	10·3	8·9	12·0	11·0	9·9	11·6	8·5	8·1	7·6											9·6	

B. *Ratios of the cross-sectional areas of the left ventricle to right ventricle in the hearts of rats from birth to the age of* 14 *days, and in the full-grown animal*

Age																						Mean ratio
At birth	0·9	1·0	1·0	1·2	1·1	1·0	1·1	1·2	1·0	1·2												1·1
1–2 days	1·1	1·3	1·5	1·1	1·3	1·2	0·9	1·3	1·7	1·2	1·7	1·0	1·7	1·3	1·7	1·2	1·3	1·4	1·4	1·2		1·3
3–4 days	2·0	1·9	2·0	1·3	2·0	1·8	1·7	2·1	1·7	1·6	2·0	1·6	1·6	2·3	2·4	2·2	2·4	2·2	1·5	2·4		1·9
5–6 days	2·5	2·6	1·8	2·2	1·9	2·0	2·5	2·0	2·1	2·3	2·5	3·0	2·6	2·6	2·5	2·0	2·0	2·0	2·7	2·3		2·3
7–8 days	2·1	2·8	2·5	2·3	2·4	2·5	2·4	2·5	2·5	3·1												2·5
9–10 days	2·4	2·1	2·5	2·9	3·5	2·7	2·8	2·6	2·7	2·6												2·7
11–12 days	2·4	2·9	3·3	2·5	3·2	3·0	3·2	2·8	2·7	2·8												2·9
13–14 days	3·3	3·1	2·6	2·9	3·3	3·2	3·3	2·8	3·0	2·8												3·0
Full grown (150 days +)	3·4	3·3	3·4	3·0	3·7	4·3	2·8	3·5	3·9	3·2												3·4

combined 3- to 4-day age group being 1·9. In the 13- to 14-day age group a mean L : R ratio of 3·0 was found. At 14 days the growing rat has roughly quadrupled its birth weight (Table 1), its eyes are open and hair has developed over the whole body. No animals older than 14 days were available for study, but it may be assumed that after this age the L : R ratio changes gradually to the figure 3·4, the mean obtained in ten hearts from full-grown rats. Individual variations of the ratios in the different groups were considerable (Table 2), but, by combining the arithmetic means of the successive age groups, a fairly regular curve was obtained (Fig. 2), demonstrating an increasing L : R ratio concomitantly with growth. The L : R ratio manifests a sharp acceleration in the first 5 days following birth, with a more gradual rise subsequently.

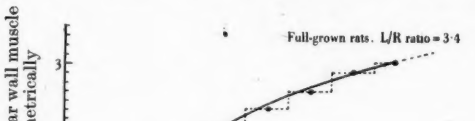

Ratio of L : R ventricular wall muscle determined planimetrically
Full-grown rats. L/R ratio = 3·4
3
2
1
At birth 1 2 3 4 5 6 7 8 9 10 11 12 13 14 days old
Age of the rats from birth to 14 days

Fig. 2. Graph showing the differential growth changes in the ventricles of the hearts of rats, from birth to 14 days.

DISCUSSION

The change in L : R ventricular ratio in the albino rat is rapid in comparison with that shown by the few specimens of infants' hearts previously recorded (Keen, 1942). This ratio is comparable with the difference in the life cycle between rat and man (Donaldson, 1924). At 3–4 days the rat's heart shows a ratio of 1·9: ratios of 2·1 and 2·4 were noted in infant hearts aged 2 and 3 months respectively. At 5–6 days the rat's heart shows a ratio of 2·3, one which is attained in the human infant before the age of 6 months. In the human heart series, specimens from infants aged 5, 6 and 12 months respectively showed an excessive thinning of the right ventricular wall and L : R ratios exceeding those of the adult heart: it remains to be established whether this represents a growth phenomenon normal to the human heart or pathological change in these particular specimens. Nothing of a comparable nature was observed in the rat series: no single specimen of the 120 hearts of rats of 14 days or less showed a ratio exceeding the 3·4 figure of the full-grown rat's heart.

SUMMARY

The results are presented of an investigation into post-natal growth changes in the cardiac ventricles of albino rats from birth to 14 days of age. Comparisons are made with observations on human infants' hearts recorded previously.

Grateful thanks are tendered to Prof. J. T. Irving for rat litters from the Department of Physiology, and acknowledgment is made of a research grant from the University of Cape Town.

REFERENCES

DONALDSON, H. A. (1924). *The Rat*, pp. 17, 173, 177. Philadelphia: Wistar Institute.
KEEN, J. A. (1942). *J. Anat., Lond.*, **77**, 104.

ATRIAL ARTERIES IN A HUMAN HEART

By W. C. OSMAN HILL, *Department of Anatomy, University of Ceylon*

In 1936 I described an atrial artery springing directly from the aorta in a Ceylonese adult elephant, and in 1938 I described a similar single vessel arising from the left coronary artery in a foetal elephant. Cave (1937) had described a single atrial artery arising from the right coronary in an Asiatic elephant.

Though Cave regarded the vessel in my earlier specimen as being anomalous and devoid of any special significance, these three cases show that there may be variation in the origin of these single atrial arteries in different species of elephant.

In the human heart it is unusual to find large single atrial arteries, and instead there are generally several small vessels arising as branches of the coronary arteries for the supply of the atria. Thus Walmsley (1929), in his account of the vessels of the heart, refers to the work of Campbell (1928), and mentions as many as four atrial branches of the right coronary artery and two of the left. Gross (1921) refers to the inconstancy of the origin of the atrial arteries.

I have therefore thought it of interest to describe the heart of a male Tamil, aged 50 years, which showed an arterial supply to the atria mainly by means of two vessels corresponding in their distribution to the single atrial artery of the elephant.

RIGHT ATRIAL ARTERY

This begins as a large, straight stem from the back of the right coronary artery 1 in. from its origin from the aorta. It passes at first upwards and to the left on the anterior surface of the right atrium and then runs more transversely in the muscular tissue of the atrium. It proceeds into the upper part of the septum, near the attachment of the latter to the atrial roof. Emerging on the back of the interatrial sulcus it sweeps round the orifice of the superior vena cava from left to right, finally reappearing on the front of the right atrium just below the caval orifice. This is the same course as that taken by the first of the four atrial vessels mentioned by Walmsley, except that in the present case the vessel is more deeply placed as it crosses the atrial roof and it surrounds the vena cava as a single vessel instead of as a plexus.

The following branches are given off by the above vessel. From its initial straight portion arise two mural branches to the anterior wall of the right atrium. The first is a long, thin artery from the deep aspect of the parent stem running at first parallel with it. It afterwards proceeds slightly to the left and ends in the adjacent muscle. The second is a slender twig from the right aspect of the parent stem. It sweeps transversely to the right and does not anastomose with the terminal circumflex caval vessels which lie above it.

The next branches arise after the emergence of the vessel on the posterior wall of the atrium. They are two in number and arise by a short common stem which springs from the parent vessel half-way down the interatrial sulcus. This stem divides at once into a recurrent and a descending branch. The recurrent vessel is the larger. It runs back in the interatrial sulcus parallel to and to the left of, but more superficial than, the main vessel. Recrossing the upper border of the atria it divides into two mural branches for the left atrium. They sweep back over the upper edge of the left atrium and supply its posterior wall. One of them, the right, descends vertically adjacent to the two right pulmonary veins. The other takes a more transverse course towards the upper left pulmonary vein.

The descending branch from the common stem occupies the interatrial sulcus as far as the opening of the inferior vena cava on the back of which it ends. It is closely related to the termination of the coronary sinus.

A slender vessel occupies the sulcus terminalis. It is a descending branch from the loop surrounding the superior caval orifice. It is completely embedded in the atrial myocardium. Below it leaves the sulcus and passes towards the right to the region of the sino-atrial node.

The distribution of the right atrial artery thus roughly corresponds with that of the first of Campbell's atrial branches of the coronary, but the following further deviations are noteworthy: (i) the lack of anastomoses between its branches or between any of these and the left atrial vessel, (ii) the recurrent course of the artery destined to supply the upper and posterior parts of the left atrium, (iii) the presence of a branch occupying the interatrial groove as well as one in the sulcus terminalis.

LEFT ATRIAL ARTERY

This is a single but smaller trunk arising from the left coronary artery a few millimetres before its division into interventricular and circumflex branches. Proceeding obliquely upwards and to the right in the general direction of the upper right pulmonary venous opening, it turns aside before

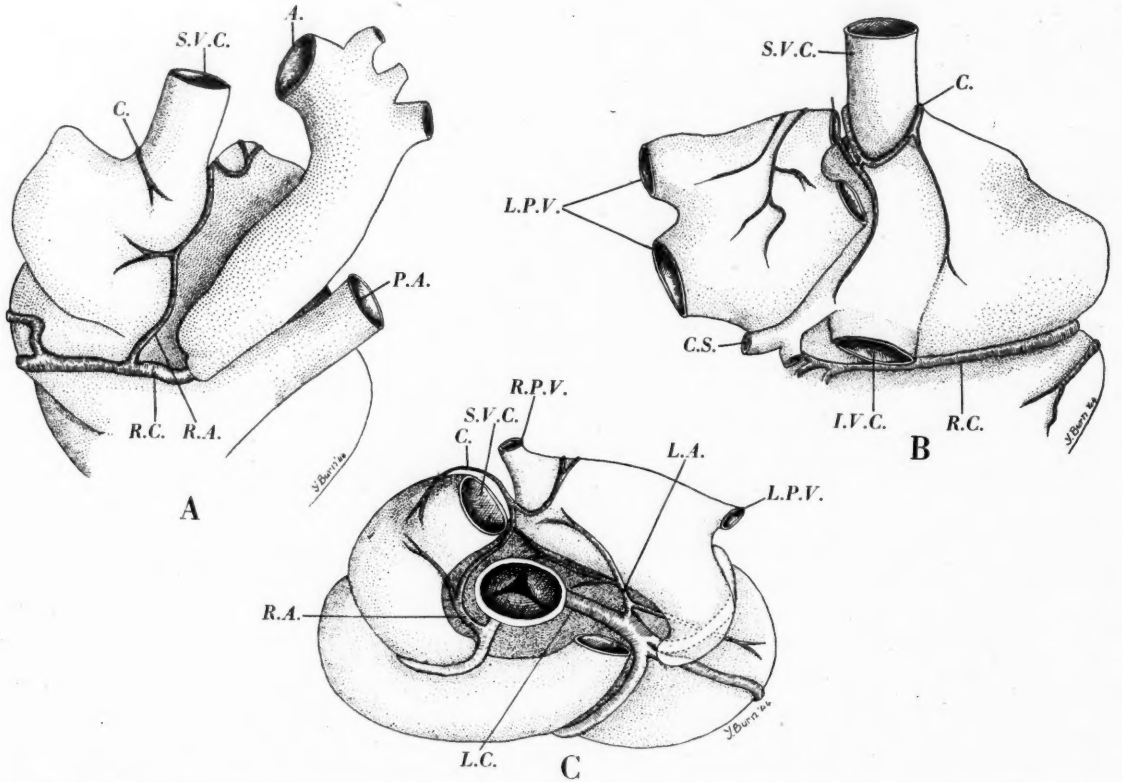


Fig 1. Atrial portion of the heart of a Tamil male, showing distribution of right and left atrial arteries from the corresponding coronary arteries. A. Ventral aspect with systemic aorta displaced to the left. B. Doral aspect. C. Cranial aspect.

Key to abbreviations: *A.* aorta; *C.* circumflex branch of right atrial artery; *C.S.* coronary sinus; *I.V.C.* inferior vena cava; *L.A.* left atrial artery; *L.C.* left coronary artery; *L.P.V.* left pulmonary veins; *P.A.* pulmonary artery; *R.A.* right atrial artery; *R.C.* right coronary artery; *R.P.V.* right pulmonary veins; *S.V.C.* superior vena cava.

meeting the left atrial branches of the right atrial artery. It gives off the following branches: (i) A moderate-sized twig arising 5 mm. from the left coronary and turning backwards towards it. It passes beneath the circumflex branch of the latter and ends in ventricular muscle in the neighbourhood of the margo obtusus. (ii) A longer vessel of similar calibre to the preceding arising as the parent vessel lies in the angle between the atrial wall and the back of the aorta. It proceeds transversely to the right in this angle and finally enters the interatrial septum. It gives off a slender twig to the posterior aortic wall.

ADDITIONAL ATRIAL ARTERIAL SUPPLY

The only other arteries traceable to the atrial walls are: (i) a small right marginal twig from the right coronary, (ii) a similar left marginal from the left coronary, (iii) a small artery from the left coronary accompanying the oblique vein of the left atrium, and (iv) a large septal artery formed by the continuation of the main stem of the right coronary. The right marginal twig crosses the coronary sulcus and divides into an atrial and a ventricular branch. The latter is additional to the large ventricular artery of the right border. The atrial portion would correspond to the second of Campbell's atrial branches from the right coronary. His remaining two vessels are unrepresented. The marginal from the left coronary is almost as large as the main left atrial. It expends itself entirely on the inferior wall of the left auricle. It is unrepresented in Campbell's scheme; his remaining atrial artery from the left coronary probably corresponds with the vessel which accompanies the oblique vein.

CONCLUSION

In conclusion, it may be stated that in the heart described the atria are supplied mainly by two large arteries, an unusually large right atrial and a somewhat smaller left. Together they are responsible for the major part of the nutrition normally controlled by six or more vessels. They also correspond in their distribution in general with the single atrial artery of the elephant's heart, though differing in detail.

It is conceivable that hearts presenting the vascular features here described may be of clinical significance especially in cases of coronary disease. Obviously such disease affecting the main coronary trunks and their early branches would receive a less favourable prognosis than that affecting the more usual type where the atrial supply is diffused through several smaller, widely spaced vessels.

REFERENCES

Campbell, J. S. (1928). The Coronary Arteries of the Heart. Thesis, Belfast; quoted by Walmsley.

Cave, A. J. E. (1937). *J. Anat., Lond.*, **71**, 124–7.

Gross, L. (1921). *The Blood Supply to the Heart.* London.

Hill, W. C. Osman (1936). *J. Anat., Lond.*, **70**, 386–98.

Hill, W. C. Osman (1938). *Ceylon J. Sci.* (B), **21**, 45–61.

Symmers, W. St C. (1907). *J. Anat. Physiol.* **41**, 141–2.

Walmsley, T. (1929). The Heart (Quain's *Anatomy*, 11th ed. vol. 4).

atrial artery; *L.C.* left coronary artery; *L.P.V.* left pulmonary veins; *P.A.* pulmonary artery; *R.A.* right atrial artery; *R.C.* right coronary artery; *R.P.V.* right pulmonary veins; *S.V.C.* superior vena cava.

BIOMETRIKA

A Journal for the Statistical Study of Biological Problems

Volume XXXIII, Part II

On autoregressive time series. By M. G. KENDALL.

Comparison of the concepts of efficiency and closeness for consistent estimates of a parameter. By R. C. GEARY.

The relation between measures of correlation in the universe of sample permutations. By H. E. DANIELS.

The growth, survival, wandering and variation of the long-tailed field mouse, *Apodemus sylvaticus*. By H. P. HACKER and HELGA S. PEARSON.

The control of industrial processes subject to trends in quality. By L. H. C. TIPPETT.

Studentization, or the elimination of the standard deviation of the parent population from the random sample-distribution of statistics. By H. O. HARTLEY.

MISCELLANEA: Note on the use of the tables of percentage points of the incomplete beta-function to calculate small sample confidence intervals for a binomial p. By HENRY SCHEFFÉ.

BOOKS RECEIVED FOR REVIEW.

The Subscription price, *payable in advance*, is 45*s*. inland, 54*s*. export, per volume (including postage). Cheques should be drawn to Biometrika and sent to "The Secretary, Biometrika Office, Department of Statistics, University College, London, W.C.1."

Issued by THE BIOMETRIKA OFFICE, University College, London

Statistical Studies in Genetics and Human Inheritance

The ANNALS OF EUGENICS

VOL. 12, PART 3 contains articles by:

MALCOLM SMITH: The families of the kings of Siam of the house of Chakri.

C. W. COTTERMAN and HAROLD F. FALLS: Genetic studies on ectopia lentis. II. Anthropometric and linkage data.

R. A. FISHER: Allowance for double reduction in the calculation of genotype frequencies with polysomic inheritance.

D. D. KOSAMBI: The estimation of map distances from recombination values.

C. H. N. JACKSON: The analysis of a tsetse-fly population. II.

R. R. RACE, G. L. TAYLOR, ELIZABETH W. IKIN and AILEEN M. PRIOR: The inheritance of allelomorphs of the *Rh* gene in fifty-six families.

Subscription, in advance, 50s. per volume

Four parts, obtainable separately at 15s. each. Back volumes, 55s. per volume.

Orders should be placed with

THE GALTON LABORATORY

c/o ROTHAMSTED EXPERIMENTAL STATION, HARPENDEN, HERTS.

VOLUME 79

CONTENTS OF PART 1

PAGE

E. GUTMANN. The reinnervation of muscle by sensory nerve fibres (with 4 Text-figures and Plates 1–4) 1

P. BACSICH AND G. M. WYBURN. The vascular pattern of peripheral nerve during repair after experimental crush injury (with Plates 1–2) 9

W. E. LE GROS CLARK AND L. B. BLOMFIELD. The efficiency of intramuscular anastomoses, with observations on the regeneration of devascularized muscle (with 7 Text-figures and Plates 1–4) 15

S. SUNDERLAND. The meningeal relations of the human hypophysis cerebri (with Plate 1) . 33

J. A. KEEN. Post-natal growth-changes in the cardiac ventricles of the albino rat (with 2 Text-figures). 38

W. C. OSMAN HILL. Atrial arteries in a human heart (with 1 Text-figure) 41

INSTRUCTIONS TO CONTRIBUTORS

1. Manuscripts should be sent to the Secretary of the Editorial Committee, Department of Human Anatomy, University Museum, Oxford.

2. Manuscripts should be typewritten or written in very legible hand and must be fully corrected. Contributors will be responsible for the payment of any sum charged for the correction of the printer's proof in excess of 10*s.* per sheet (16 pages).

3. Illustrations must be in a finished form ready for reproduction. They must be properly lettered or labelled in type or by hand with reference pointers if necessary.

4. Line drawings must be sent whenever possible. Illustrations requiring half-tone blocks are very costly, *particularly under the present circumstances.*

5. The legend describing an illustration must be inserted in its appropriate place in the text and should not be placed on or appended to the drawing.

6. A contribution must terminate with a brief summary of the chief descriptions or observations and any conclusions drawn therefrom. The author is requested to provide the Editor with a type-written abstract of his contribution not exceeding 3% of the text for publication in *Biological Abstracts*.

7. References are to be arranged in alphabetical order of the authors' names at the end of the contribution, the date of the paper being placed in brackets immediately after the author's name. Abbreviations for titles of journals given in the *World List of Scientific Periodicals* should be used. Subjects titles should be omitted. In the text a reference to a publication should be cited by giving the author, and, in brackets, the date, thus: "Allis (1934) found, etc."

8. The Editorial Committee cannot accept responsibility for damage to or loss of papers submitted to them. Contributors are advised to keep an additional copy of the manuscript and photographic reproductions of the illustrations.

9. Fifty gratis copies are given to each contributor.

10. The Journal is issued at the price of £2. 0*s.* 0*d.* per volume. Subscriptions, payable in advance, should be sent to the Cambridge University Press, Bentley House, 200 Euston Road, London, N.W. 1.

PRINTED IN GREAT BRITAIN BY W. LEWIS, M.A., AT THE UNIVERSITY PRESS, CAMBRIDGE